ATENT COOPERATION TREATY

	From the INTERNATIONAL BUREAU
PCT	To:
	Commissioner
NOTIFICATION OF ELECTION (PCT Rule 61.2)	US Department of Commerce United States Patent and Trademark Office, PCT 2011 South Clark Place Room CP2/5C24 Arlington, VA 22202 ETATS-UNIS D'AMERIQUE
Date of mailing (day/month/year) 22 May 2001 (22.05.01)	in its capacity as elected Office
International application No. PCT/EP00/07835	Applicant's or agent's file reference K 2840 Wd
International filing date (day/month/year) 11 August 2000 (11.08.00)	Priority date (day/month/year) 13 August 1999 (13.08.99)
Applicant	
NÜESCH, Jürg et al	
The designated Office is hereby notified of its election made in the demand filed with the International Preliminary 10 March 2001	Examining Authority on:
in a notice effecting later election filed with the Intern	
2. The election X was	
was not	
made before the expiration of 19 months from the priority di Rule 32.2(b).	ate or, where Rule 32 applies, within the time limit under
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The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Authorized officer

Claudio Borton

(19) World Intellectual Property Organization International Rureau



(43) International Publication Date 22 February 2001 (22.02.2001)

PCT

(10) International Publication Number WO 01/12666 A1

C07K 14/015 (51) International Patent Classification7:

[CH/CH]: In den Wegscheiden 1, CH-4132 Muttenz (CH). ROMMELAERE, Jean [BE/DE]; Schloss Wolfsbrunneaweg 11, D-69118 Heidelberg (DE).

eringer Strasse 246, D-81825 München (DE).

- (21) International Application Number: PCT/EP00/07835
- (74) Agent: SCHÜSSLER, Andrea; Huber & Schüssler, Trud-(22) International Filing Date: 11 August 2000 (11.08.2000)
- (25) Filing Language:

English English

- (26) Publication Language: (30) Priority Data: 99115161.4
 - 13 August 1999 (13.08.1999) EP
- (71) Applicant (for all designated States except US): DEUTSCHES KREBSFORSCHUNGSZENTRUM [DE/DE]; Stiftung des öffentlichen Rechts, Im Neuenheimer Feld 280, D-69120 Heidelberg (DE).
- Published:
- (81) Designated States (national): JP, US. With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.
- With (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description.

(72) Inventors; and

(75) Inventors/Applicants (for US only): NÜESCH, Jürg ning of each regular issue of the PCT Gazette.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the begin-

(54) Title: PARVOVIRUS NS1 VARIANTS

(57) Abstract: The present invention relates to a parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b). Furthermore, this invention relates to DNAs coding for these parvovirus NS1 variants and methods of producing them. Additionally, this invention concerns antibodies directed against the parvovirus NS1 variants as well as the use of the DNAs and the parvovirus NS1 variants.

	DCT	For rea	ceiving Office use only		
PCT					
		International Application I	No.		
	REQUEST	International Filing Date			
		Tank and the same			
	The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.	Name of receiving Office	and "PCT International Application"		
		Applicant's or agent's file (if desired) (12 characters i			
	Box No. I TITLE OF INVENTION				
	Parvovirus NS1 Variants				
	Box No. II APPLICANT				
	Name and address: (Family name followed by given name; for a designation. The address must include postal code and name of cox address indicated in this Box is the applicant's State (that is, country of residence is indicated below.)	legal entity, full official arry. The country of the a) of residence if no State	This person is also inventor.		
(Tetebuoue 140.		
	Deutsches Krebsforschungszentru Stiftung des öffentlichen Recht: Im Neuenheimer Feld 280	П 5	Facsimile No.		
	69120 Heidelberg		Teleprinter No.		
	State (that is, country) of nationality: DE	State (that is, country) o			
	This person is applicant all designated all designated for the purposes of:	ed States except States of America of	e United States the States indicated in the Supplemental Box		
	Box No. III FURTHER APPLICANT(S) AND/OR (FURT				
	Name and address: (family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's Stare (that is, country) of residence if indicated below.] This person is: applicant only				
()	Nüesch, Jürg In den Wegscheiden 1		x applicant and inventor inventor only (If this check-box is marked, do not fill in below.)		
	CH-4132 Muttenz	State (that is, country)	of moldonae		
	State (that is, country) of nationality:	CH			
	This person is applicant all designated all designated for the purposes of:	ted States except States of America	the United States the States indicated in the Supplemental Box		
	Further applicants and/or (further) inventors are indicated on a continuation sheet.				
	BOX NO. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE				
	The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:				
	Name and address: (Family name followed by given name; for designation. The address must include postal	a legal ensity, full official code and name of country.)	Telephone No.		
	Dr. Andrea Schüßler	austra ex ESS	Facsimile No.		
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	Address for correspondence: Mark this check-box where space above is used instead to indicate a special address to	no agent or common repre	sentative is/has been appointed and the		
	Form PCT/RO/101 (first sheet) (July 1998)		See Notes to the request form		

Sheet No2				
Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) IN	VENTOR(S)			
If none of the following sub-boxes is used, this sheet should not be inc	luded in the request.			
Name and address, (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address subtracted in this Box is the applicant's State (that is, country) of residence if no State of Postatical Country of the applicant's State (that is, country) of residence if no State of Postatical Country of the applicant of th	This person is:			
Fommelaere, Jean Schloß Wolfsbrunnenweg 11 D-69118 Heidelberg State (that is, country) of madonality: B State (that is, country)	applicant only applicant and inventor inventor only (If this check-hox is marked, do not fill in below) of residence: United States the States indicated in America only the Supplemental Box This person is: applicant only applicant only inventor only (If this check-box			
State (that is, country) of nationality: State (that is, country) of nationality: State (that is, country) State (that is, country) This person is applicant Composition of the person of the per	is marked, do not fill in below.)			
for the purposes of: States	This person is: applicant only applicant and inventor inventor only [If this check-box is marked, do not fill in below.]			
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This person is applicant all designated all designated States except for the purposes of:	the United States indicated in the States indicated in the Supplemental Box			
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State (that is, country) of nationality: State (that is, country) of residence:				
This person is applicant for the purposes of: all designated States except the United States of America	the United States of America only the States indicated in the Supplemental Box			
Further applicants and/or (further) inventors are indicated on another continuation sheet.				

Sheet No. 3

Box No.V	DESIGNATION OF STATES				
The following	The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes: at least one must be marked):				
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SZ Sv of the	waziland, TZ United Republic of Tanzania, UG Ugan Harare Protocol and of the PCT	ıda,	ZW:	Zimbabwe, and any other State which is a Contracting State	
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Precautionary	Designation Statement: In addition to the designation	ion	mad	c above, the applicant also makes under Rule 4.9(b) all other	
designations wi	designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded				
from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any					

designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)

Date of receipt of by the Internations

Form PCT/RO/101 (last sheet) (July 1998)

Sheet No. 4 Further priority claims are indicated in the Supplemental Box. Box No. VI PRIORITY CLAIM Where earlier application is: Number Filing date of carlier application regional application:* international application: of earlier application (day/month/year) national application: receiving Office regional Office country item (1) EPO 99 115 161.4 13. 1999 201 15 15 item (2) item (3) The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s): 1 Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Parts overention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(fi)). See Supplemental Box. BOX No. VII INTERNATIONAL SEARCHING AUTHORITY Choice of International Searching Authority (ISA) (if two or more International Searching Authorities or competent to carry out the international tearch, indicate the Authority chosen; the two-letter code may be used); Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority): Country (or regional Office) Date (day/month/year) ISA / EP BOX NO. VIII CHECK LIST; LANGUAGE OF FILING This international application is accompanied by the item(s) marked below: This international application contains the following number of sheets: 1. VI fee calculation sheet request 2. Separate signed power of attorney 10 description (excluding copy of general power of attorney; reference number, if any: sequence listing part) 2 d. ☐ statement explaining lack of signature claime priority document(s) identified in Box No. VI as item(s); abetract 1 translation of international application into (language); drawings 7. separate indications concerning deposited microorganism or other biological material 27 sequence listing part of description 8. 🗖 nucleotide and/or amino acid sequence listing in computer readable form 9. other (specify): Total number of sheets: Language of filing of the Figure of the drawings which international application: should accompany the abstract: BOX NO. IX SIGNATURE OF APPLICANT OR AGENT Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request) München, 10. Aug. 2000 Dr. Andrea Schüßler European Patent Attorney - For receiving Office use only 2. Drawings: Date of actual receipt of the purported international application: received: Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application: not received: Date of timely receipt of the required corrections under PCT Article 11(2); 5. International Searching Authority ISA / Transmittal of search copy delayed until search fee is paid. (if two or more are competent):

	For International Bureau use only	
he record copy I Burcau:		
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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

	(FOT PARTIES TO STATE TO STATE TO				
Applicant's or agent's file reference K 2840 Wd	(Form PCT/ISA/220) as well as, where applicable, item 5 below.				
International application No.	International filing date (day/month/year)	(Earliest) Priority Date (day/month/year)			
PCT/EP 00/07835	11/08/2000	13/08/1999			
Applicant					
DEUTSCHES KREBSFORSCHUNGS	ZENTRUM et al.	·			
This International Search Report has bee according to Article 18. A copy is being to	n prepared by this International Searching Aut ansmitted to the International Bureau.	hority and is transmitted to the applicant			
This international Search Report consists It is also accompanied by	of a total ofsheets. a copy of each prior art document cited in this	report.			
1. Basis of the report					
a. With regard to the language, the	International search was carried out on the ba	isis of the International application in the			
	less otherwise indicated under this item.	and the second s			
Authority (Rule 23.1(b)).	vas carried out on the basis of a translation of				
 b. With regard to any nucleotide at was carried out on the basis of th 	nd/or amino acid sequence disclosed in the i	nternational application, the international search			
	onal application in written form.				
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the statement that the su international application	bsequently furnished written sequence listing as filed has been furnished.	does not go beyond the disclosure in the			
the statement that the Informished	formation recorded in computer readable form	is identical to the written sequence listing has been			
2. X Certain claims were for	und unsearchable (See Box I).				
3. Unity of invention is la	cking (see Box II).	-			
4. With regard to the title,	shmitted by the applicant				
	the text is approved as submitted by the applicant.				
the text has been established by this Authority to read as follows:					
5. With regard to the abstract,					
X the text is approved as submitted by the applicant.					
the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.					
The figure of the drawings to be published with the abstract is Figure No.					
as suggested by the app	plicant	None of the figures.			
because the applicant failed to suggest a ligure.					
because this figure better characterizes the Invention.					

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International application No. PCT/EP 00/07835

INTERNATIONAL SEARCH REPORT

Box Observations wh re certain clalms were found unsearchable (C ntinuation of item 1 of first sh et)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely: Although claims 14 and 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the NSI variant. 2. Claims Nos: because they relate to parts of the international Application that do not comply with the prescribed requirements to such an exent that no meaningful informational Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a). Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This International Searching Authority found multiple Inventions in this international application, as follows:
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
No required additional search fees were timely paid by the applicant. Consequently, this International Search Report Is restricted to the Invention first mentioned in the claims; It is covered by claims Noc.:
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

Internacional	Application No
PCT/EP	00/07835

		1 1 1 10	707033	
A. CLASSIF IPC 7	CATIONOF SUBJECT MATTER C12N15/35 C07K14/015 C07K16/0 A61K35/76 A61K48/00	8 G01N33/569 C12Q	1/70	
According to	International Patent Classification (IPC) or to both national classifica	lion and IPG		
B. FIELDS	SEARCHED			
Minimum do	cumentation searched (classification system followed by classification C12N C07K A61K			
	on searched other than minimum documentation to the extent that so	_		
Electronic da	ata base consulted during the International search (name of data bas	se and, where practical, search terms use	າ	
EPO-In	ternal, STRAND, MEDLINE, BIOSIS			
C. DOCUME	ENTS CONSIDERED TO BE RELEVANT			
Category *	Citation of document, with indication, where appropriate, of the rele	evant passages	Relevant to claim No.	
X D LEGENDRE & J ROMMELARE: "Terminal regions of the NS-1 protein of the parvovirus Minute Virus of Mice are involved in cytotoxicity and promoter trans inhibition" JOURNAL OF VIROLOSY, vol. 66, no. 10, October 1992 (1992-10), pages 5705-5713, XPO00867510 AMERICAN SOCIETY FOR MICROBIOLOGY US *mutants pMMBal31 and pULB3201; figure 1 and page 5710 first paragraph* -/				
X Fun	her documents are fisted in the continuation of box C.	Patent family members are liste	d in annex.	
*Special categories of olled documents: *A' document defining the general state of the art which is not considered to be of particular relevance *E' earlier document be application and increased to be of particular relevance *L' document which may show doubts on priority dafris() or which is cold to adhabit the publication shall of arthority dafris() or which is cold to adhabit the publication shall of arthority dafris() or which is cold to adhabit the publication shall not of arthority dafris() or which is cold to adhabit the publication shall not increased to a state of the publication shall not adhabit to a state of the publication of the state of a state of the publication of the state of the same of the state of the				
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TIONAL SEARCH REPORT

PCT/EP 00/07835

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C.(Contin	C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT Output as for your provide and the relevant passages. Relevant to claim No.					
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relev	uni io citim No.			
A	LI X ET AL: "Mutation of lysine 405 to serine in the parvovirus H-1 NS1 abolishes		1,3,7, 9-11			
	its function for viral DNA replication, late promoter activation, and cytotoxocity" JOURNAL OF VIROLOGY, vol. 64, no. 10, October 1990 (1990-10), pages 4654-4660, XPO00867496 AMERICAN SOCIETY FOR MICROBIOLOGY US page 4656 -page 4657					
	J P F NÜESCH ET AL: "Sequence motifs in the replicator protein of parvovirus MVM essential for nicking and covalent attachement to the viral origin: identification of the linking tyrosine" VIROLOGY, US, ACADEMIC PRESS, ORLANDO, vol. 209, no. 1, 10 May 1995 (1995-05-10), pages 122-135, XPO02088311 ISSN: 0042-6822 page 127 -page 131		1-13			
. A	S F COTTMORE ET AL: "The NS1 polypeptide of the murine parvovirus MVM binds to DNA sequences containing the motif (ACCA)2-3" JOURNAL OF VIROLOGY, US, THE AMERICAN SOCIETY FOR MICROBIOLOGY, Vol. 69, no. 3, pages 1652-1660, XP002088309 ISSN: 0022-538X page 1658, left-hand column, last paragraph -right-hand column		1-13			
1						

The demand must be filed directly with				
with the one chosen by the applicant.	The full name or two-lette	r code of that Authority may l	be indicated by the applicant	on the line below:
IPEA/				

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For	International Preliminar	y Examining Authority	use only	
Identification of IPEA		Date of receipt of DEMAND		
Box No. I IDENTIFICATION OF THE INTERNATIONAL API		APPLICATION	Applicant's or agent's file reference K 2840 Wd	
International application No.	International filing dat	c (day/month/year)	(Earliest) Priority date (day/month/year)	
PCT/EP00/07835	Aug. 11, 2000		Aug. 13, 1999	
Title of invention				
Parvovirus NS1 Variants	•			
Box No. II APPLICANT(S)			9	
Name and address: (Family name followed by The address must include p	given name; for a legal entity, costal code and name of countr	full official designation. 9.)	Telephone No.:	
Deutsches Krebsforschur Stiftung des öffentlich	en Rechts		Facsimile No.:	
Im Nevenheimer Feld 280 69120 Heidelberg			Teleprinter No.:	
State (Le. country) of nationality:		State (i.e. country) o	te (i.e. country) of residence:	
DE			DE	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) NÜESCH, JÜrg In den Negscheiden 1 CH-4132 Muttenz				
State (i.e. country) of nationality:		State (i.e. country) of residence:		
ОН			СН	
Name and address: (Partily name followed by given name: for a legal ently, full official destination. The address must include period code and name of country.) ROMMELAERE, Jean Schillaß Wolfsbrunnenweg 11 D-69118 Heidelberg				
State (i.e. country) of nationality:		State (i.e. country) o	f residence: DE	
Further applicants are indicated on a continuation sheet.				

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE The following person is agent common representative and as been appointed earlier and representative appointment of (an) agent(s)/common representative is hereby appointed. is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked. is hereby appointed, specifically for the procedure before the International preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier. Name and address: (Family name followed by pine name; for a legal entity, full affected designation. Dr. Andrea Schüßler HÜBER & SCHÜSSLER Patantanville: Pritein Autoritye Thickefrings Trinke 24-18 (1882) Minghen Teleptone No: Presimile No: Presimile No: Presimile No: Presimile No: Presimile No: International application No. Presimile No: P			
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and has been appointed earlier and represents the applicant(s) also for international preliminary examination. is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked. is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative applicant dearlier. Name and address: [Family agent(s)/common representative applicant dearlier. Name and address: [Family agent(s)/common representative applicant dearlier. Dr. Andrea Schüßler HUBER & SCHÜSSLER Facination Faci	Box No. III	AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CO	RRESPONDENCE
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For International	Preliminary Exa	mining Authority us	e only
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From the			
INTERNATIONAL	PRELIMINARY	FYAMINING	AUTHORITY

PCT To: Hober & Schüßler SCHOSSILER: Angrea HUBER & SCHÜSSLER NOTIFICATION OF TRANSMITTAL OF Patentanwälte Truderinger Strasse 246 THE INTERNATIONAL PRELIMINARY D-81825 München 0 3. DEZ. 2001 **EXAMINATION REPORT** ALLEMAGNE (PCT Rule 71.1) Date of mailing 29.11.2001 (day/month/year) Applicant's or agent's file reference IMPORTANT NOTIFICATION K 2840 Wd International filing date (day/month/year) Priority date (day/month/year) International application No. 13/08/1999 11/08/2000 PCT/EP00/07835 Applicant DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.

- 1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
- 2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
- 3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4 REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the International application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

Fax: +31 70 340 - 3016

Authorized officer Cardenas, C

European Palent Office - P.B. 5818 Patentiaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl

Tel.+31 70 340-3370

Form PCT/IPEA/416 (July 1992)



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or ager	nt's file reference	FOR FURTHER ACT	See Notifica	uton of Transmittal of International Examination Report (Form PCT/IPEA/416)				
K 2840 Wd		FOR FORTHER ACT	TOTA Premimary					
International applic	ation No.	International filing date (da	y/month/year)	Priority date (day/month/year)				
PCT/EP00/078	PCT/EP00/07835 11/08/2000 13/08/1999							
International Pater C07K14/015	nt Classification (IPC) or na	tional classification and IPC						
Applicant								
DEUTSCHES	KREBSFORSCHUNG	GSZENTRUM et al.						
and is trans 2. This REPO This re been a (see R	mitted to the applicant a RT consists of a total of port is also accompanies mended and are the ba	according to Article 36. 6 Sheets, including this od by ANNEXES, Le. sheets for this report and/or a coro of the Administrative I	cover sheet. ets of the descriptionsheets containing re	mational Preliminary Examining Authority n, claims and/or drawings which have citications made before this Authority to PCT).				
	Basis of the report Priority Non-establishment of Lack of unity of invent Reasoned statement ic citations and explanat Certain documents of Certain defects in the	ion under Article 35(2) with re lions suporting such state	velly, inventive step gard to novelly, inv ment	and industrial applicability entive step or industrial applicability;				
Date of submissi	on of the demand		Date of completion of	f this report				

Date of submission of the demand	Date of completion of this report	
10/03/2001	29,11.2001	
Name and mailing address of the international preliminary examining authority:	Authorized officer	A STATE OF THE PARTY OF THE PAR
European Patent Office - P.B. 5816 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl	Cupido, M	
Fax: +31 70 340 - 3016	Telephone No. +31 70 340 3374	700.00

International application No. PCT/EP00/07835

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		of the report				
	the re and a	anising Office In	nents of the international ap response to an invitation un o this report since they do n	der Article 14 are .	referred to in this re	eport as originally illeu
	1-10		as originally filed			
	Clair	ns, No.:				
	14,1	5	as originally filed			
	1-13		as received on	06/11/2001	with letter of	06/11/2001
	Drav	vings, sheets:				
	1/6-6	8/6	as originally filed			
2.	With lang	regard to the lan	guage, all the elements ma international application wa	rked above were a as filed, unless oth	available or furnish erwise Indicated ui	ed to this Authority in the nder this item.
	The	se elements were	available or fumished to thi	s Authority in the f	following language:	, which is:
			translation furnished for the			h (under Rule 23.1(b)).
		the language of p	publication of the internation	al application (und	der Rule 48.3(b)).	
		the language of a 55.2 and/or 55.3)	a translation furnished for th	e purposes of inte	rnațional prelimina	ry examination (under Rule
3.	With	n regard to any nu mational prelimina	icleotide and/or amino aci ary examination was carried	d sequence discless of the desired on the basis of	osed in the internat of the sequence lis	tional application, the ting:
		contained in the	International application in v	vritten form.		
			h the international application		dable form.	
		furnished subsec	quently to this Authority in w	ritten form.		
			quently to this Authority in co		form.	
		The statement the	nat the subsequently furnish application as filed has bee	ed written sequen n furnished.	ce listing does not	go beyond the disclosure in
		The statement the listing has been	nat the information recorded furnished.	in computer read	able form is identic	al to the written sequence
4	. The	amendments ha	ve resulted in the cancellation	on of:		

International application No. PCT/EP00/07835

		the description,	pages:
		the claims,	Nos.:
		the drawings,	sheets:
5,	0	This report has been considered to go bey	established as if (some of) the amendments had not been made, since they have been youd the disclosure as filed (Rule 70.2(c)):
		(Any replacement st report.)	neet containing such amendments must be referred to under Item 1 and annexed to this
6.	Add	itional observations,	if necessary:
111	Nor	-establishment of o	pinion with regard to novelty, inventive step and industrial applicability
	The	questions whether th	ne claimed invention appears to be novel, to involve an inventive step (to be non- ially applicable have not been examined in respect of:
		the entire internation	nal application.
	×	claims Nos. 12,13.	
be	ecaus	se:	
	×	the said internationa does not require an see separate sheet	al application, or the said claims Nos. 12,13 relate to the following subject matter which international preliminary examination (specify):
			ms or drawings (indicate particular elements below) or said claims Nos. are so unclear opinion could be formed (specify):
		the claims, or said o	claims Nos. are so inadequately supported by the description that no meaningful opinion
		no international sea	urch report has been established for the said claims Nos
2	and	neaningful internation d/or amino acid seque tructlons:	nal preliminary examination cannot be carried out due to the failure of the nucleotide ence listing to comply with the standard provided for in Annex C of the Administrative
		the written form has	s not been furnished or does not comply with the standard.
		the computer reada	ible form has not been furnished or does not comply with the standard.
٧			under Article 35(2) with regard to novelty, inventive step or industrial applicability;



International application No. PCT/EP00/07835

1. Statement

Novelty (N)

Yes: Claims 4, 10, 11

No: Claims 1-3, 5-9

Inventive step (IS)

Yes: Claims No: Claims 1-11

Industrial applicability (IA)

Yes: Claims 1-11

No: Claims

2. Citations and explanations see separate sheet

VIII, Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made: see separate sheet

INTERNATIONAL PRELIMINARY International application No. PCT/EP00/07835 EXAMINATION REPORT - SEPARATE SHEET

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 12 and 13 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(l) PCT).

For the assessment of these claims on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability: citations and explanations supporting such statement

I Document

The following document has been taken into consideration:

D1: J. Virol. 66, 5705-5713 (Legendre and Rommelaere, 1992)

II Novelty

D1 discloses a number of mutants in the NS1 protein of the parvovirus MVM. According to figure 1, mutants pMMBa131 and pULB3201 are still of cytotoxicity class I, showing that cytotoxicity is maintained although at a reduced level, but still regarded as being highly toxic, see page 5709 last paragraph, whereas P38 transactivation and DNA replication are strongly inhibited. pMMBa131 contains a deletion of amino acids 245-313, pULB3201 produces an NS1 with a deletion of 374 amino acids (from positions

INTERNATIONAL PRELIMINARY International application No. PCT/EP00/07835 EXAMINATION REPORT - SEPARATE SHEET

167 to 547), and these mutations are therefore located at the positions referred to in claim 5. Consequently, there can be no doubt that D1 describes NS-1 mutants and their nucleic acids with the characteristics of the NS-1 mutants and DNAs claimed in claims 1-3 and 5-9, and these claims lack novelty in view of Article 33(2) PCT.

III Inventive step

- 1. D1 is regarded as the closest prior art with respect to the question whether the claimed subject-matter involves an inventive step. The problem underlying the present application in view of D1 is the provision of further parvovirus NS1 proteins that can be used as a toxin for treating tumoral diseases.
- 2. The novel solutions to this problem provided and claimed in claim 4 of the present invention consist of four specific NS1 variants, designated as S283A, T363A, T394A and T463A. According to Table 1, these NS1 variants with the exception of T463A, are still cytotoxic, whereas P38 transactivation and DNA replication are strongly reduced. These variants can be regarded as possible candidates for use as a toxin in antitumour treatments. Hence, the subject-matter regarding NS1 variants, S283A, T363A and T394A is regarded to involve an inventive step as required by Article 33(3) PCT. Subject-matter relating to the non-toxic NS1 variant T463A is regarded not to involve an inventive step.
- 3. The antibody and kit referred to in claims 10 and 11 are characterised by the protein sequences to which these antibodies are directed. Since these antigen sequences are known from D1, and antibodies directed to known antigens are devoid of an inventive step, claims 10 and 11 also do not involve an inventive step and these claims cannot be accepted in view of Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

Claim 6 refers to DNA coding for parvovirus NS1 variants having the following phosphorylation site mutants: S283A, T363A, T394A, or T463A, wherein the DNA comprises the DNA of figure 1. The DNA of figure 1 represents the wild-type NS1. Hence, the claim is contradictory and violates the requirements of Article 6 PCT.







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Claims

- A parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities
 (a) and the cytotoxicity activity (b), wherein
- 5 the activities (a) are reduced and eliminated, respectively, and activity (b) is maintained or increased or
- activity (b) is reduced and eliminated,
 respectively, and the activities (a) are maintained or increased.
 - The parvovirus NS1 variant according to claim 1, wherein one or several phosphorylation sites are mutated.
 - The parvovirus NS1 variant according to claim 2, wherein the mutations are located at sites 283, 363, 394 and/or 463.
 - The parvovirus NS1 variant according to claim 2 or 3, namely the NS1 variants S283A, T363A, T394A, and T463A.
 - A DNA, coding for the parvovirus NS1 variant according to any one of claims 1 to 4.
 - 6. The DNA according to claim 5, wherein the DNA comprises:
 - (a) the DNA of figure 1,
 - (b) a DNA hybridizing with the DNA from (a), said DNA comprising the mutated phosphorylation site of the DNA from (a), or
 - (c) a DNA related to the DNA from (a) or (b) via the degenerated genetic code.
 - 35 7. An expression vector, comprising the DNA according to





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claim 5 or 6.

 A transformant, containing the expression vector according to claim 7.

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- 5
- A method of producing the parvovirus NS1 variant according to any one of claims 1 to 4, comprising the culturing of the transformant according to claim 8 under suitable conditions.

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 An antibody, directed against the parvovirus NS1 variant according to any one of claims 1 to 4.

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11. Kit comprising:

15 (a) a parvovir

- (a) a parvovirus NS1 variant according to the invention,
- (b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,
- (c) an antibody according to the invention, as well as
- (d) conventional auxiliary agents, such as solvents, buffers, carriers, markers and controls,

wherein of components (a) to (d) one or more representatives can be present each.

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- 12. Use of the parvovirus NS1 variant according to any one of claims 1 to 4 as a toxin for treating tumoral diseases.
 - Use of the DNA according to claim 7 as a vector for gene therapy.

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Claims

- A parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b).
- The parvovirus NS1 variant according to claim 1, wherein the activities (a) are reduced and eliminated, respectively, and activity (b) is maintained or increased.
- The parvovirus NS1 variant according to claim 1, wherein activity (b) is reduced and eliminated, respectively, and the activities (a) are maintained or increased.
- The parvovirus NS1 variant according to any one of claims 1 to 3, wherein one or several phosphorylation sites are mutated.
- The parvovirus NS1 variant according to claim 4, wherein the mutations are located at sites 283, 363, 394 and/or 463.
- The parvovirus NS1 variant according to claim 4 or 5, namely the NS1 variants S283A, T363A, T394A, and T463A.
- A DNA, coding for the parvovirus NS1 variant according to any one of claims 1 to 6.
- 8. The DNA according to claim 7, wherein the DNA comprises:
 - (a) the DNA of figure 1,
 - (b) a DNA hybridizing with the DNA from (a), said DNA comprising the mutated phosphorylation site of the DNA from (a), or
 - (c) a DNA related to the DNA from (a) or (b) via the degenerated genetic code.

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- An expression vector, comprising the DNA according to claim 9. 7 or 8.
- 10. A transformant, containing the expression vector according to claim 9.
- 11. A method of producing the parvovirus NS1 variant according to any one of claims 1 to 6, comprising the culturing of the transformant according to claim 10 under suitable conditions.
- 12. An antibody, directed against the parvovirus NS1 variant according to any one of claims 1 to 6.
- Kit comprising: 13.
 - (a) a parvovirus NS1 variant according to the invention,
 - (b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,
 - (c) an antibody according to the invention, as well as
 - (d) conventional auxiliary agents, such as solvents, buffers, carriers, markers and controls,

wherein of components (a) to (d) one or more representatives can be present each.

- 14. Use of the parvovirus NS1 variant according to any one of claims 1 to 6 as a toxin for treating tumoral diseases.
- 15. Use of the DNA according to claim 9 as a vector for gene therapy.

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The present invention relates to a parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b). Furthermore, this invention relates to DNAs coding for these parvovirus NS1 variants and methods of producing them. Additionally, this invention concerns antibodies directed against the parvovirus NS1 variants as well as the use of the DNAs and the parvovirus NS1 variants.



PCT

REC'D 3 0 NOV 2001

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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	or agent's file reference	FOR FURTHER ACT		otification of Transmittal of International
K 2840 W	d	FOR FURTHER ACT	Prelimi	nary Examination Report (Form PCT/IPEA/416)
Internationa	application No.	International filing date (da	y/month/year)	Priority date (day/month/year)
PCT/EP0	0/07835	11/08/2000		13/08/1999
Internationa C07K14/0		or national classification and IPC		
Applicant			•	
DEUTSC	HES KREBSFORSCH	UNGSZENTRUM et al.		
		xamination report has been praint according to Article 36.	epared by this	International Preliminary Examining Authorit
2. This F	EPORT consists of a tot	al of 6 sheets, including this of	over sheet.	
b	en amended and are th		heets containin	ption, claims and/or drawings which have g rectifications made before this Authority er the PCT).
,				
These	annexes consist of a tol	al of 2 sheets.		
1 11 111	☒ Basis of the report☐ Priority☒ Non-establishmen	t of opinion with regard to nove		tep and industrial applicability
IV	☐ Lack of unity of inv			
V		ent under Article 35(2) with reg inations suporting such staten		inventive step or industrial applicability;
VI	☐ Certain document			
VII	☐ Certain defects in	the international application		
VIII	□ Certain observation	ns on the international applica	tion	
Date of sub	nission of the demand		Date of completion	n of this report
			·	•
10/03/200	1		29.11.2001	
	nalling address of the intern	ational	Authorized officer	AND ES MODES
preliminary	examining authority: European Patent Office - F	.B. 5818 Patentlaan 2		
<i>)</i>))	NL-2280 HV Rijswijk - Pay Tel. +31 70 340 - 2040 Tx		Cupido, M	(<u>y</u>
	Fax: +31 70 340 - 3016		Telephone No. +3	31 70 340 3374

International application No. PCT/EP00/07835

I.	Basis of	the rep	ort		

		receiving Office in	response to an invitation under o this report since they do not co	Article 14 are	referred to in this rep	ort as "originally filed"
	Des	cription, pages:				
	1-10	0	as originally filed			
	Cla	ims, No.:				
	14,1	15	as originally filed			
	1-13	3	as received on	06/11/2001	with letter of	06/11/2001
	Dra	wings, sheets:				
	1/6-	6/6	as originally filed			
2.			guage, all the elements marked international application was file			
	The	se elements were	available or furnished to this Aut	thority in the f	ollowing language:	which is:
		the language of a	translation furnished for the pur	poses of the i	nternational search (ı	ınder Rule 23.1(b)).
		the language of pr	ublication of the international ap	plication (und	er Rule 48.3(b)).	
		the language of a 55.2 and/or 55.3).	translation furnished for the pur	poses of inter	national preliminary e	xamination (under Rule
3.			cleotide and/or amino acid sec ry examination was carried out o			
		contained in the in	nternational application in writter	form.		
		filed together with	the international application in o	computer read	lable form.	
		furnished subsequ	ently to this Authority in written	form.		
		furnished subsequ	uently to this Authority in compu-	ter readable f	orm.	
			at the subsequently furnished wr application as filed has been furn		e listing does not go	peyond the disclosure in
		The statement that listing has been fu	at the information recorded in co urnished.	mputer reada	ble form is identical to	the written sequence
4.	The	amendments have	e resulted in the cancellation of:			

		the description,	pages:			
		the claims,	Nos.:			
		the drawings,	sheets:			
5.			n established as if (som yond the disclosure as			e, since they have beer
		(Any replacement si report.)	heet containing such ar	mendments must be	referred to under iten	n 1 and annexed to this
6.	Add	litional observations,	if necessary:			
111.	. Noi	n-establishment of c	pinion with regard to	novelty, inventive	step and industrial a	applicability
1.			ne claimed invention ap rially applicable have no nal application.			step (to be non-
	⊠	claims Nos. 12,13.				
b€	caus	se:				
	Ø		al application, or the sai international preliminar t			ı subject matter which
			ms or drawings (<i>indicat</i> opinion could be formed		<i>ts below</i>) or said claim	s Nos. are so unclear
		the claims, or said could be formed.	laims Nos. are so inad	lequately supported	by the description that	ut no meaningful opinior
		no international sea	rch report has been est	tablished for the sai	d claims Nos	
2.	and		al preliminary examinatence listing to comply w			
		the written form has	not been furnished or	does not comply wi	th the standard.	
		the computer reada	ble form has not been f	urnished or does n	ot comply with the stan	ndard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;

International application No. PCT/EP00/07835

Form PCT/IPEA/409 (Boxes I-VIII, Sheet 2) (July 1998)

citations and explanations supporting such statement

International application No. PCT/EP00/07835

1. Statement

Novelty (N)	Yes: No:		4, 10, 11 5 1-3, 5-9
Inventive step (IS)	Yes: No:	Claims Claims	
Industrial applicability (IA)	Yes: No:	Claims Claims	

2. Citations and explanations see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made: see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 12 and 13 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

For the assessment of these claims on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

I Document

The following document has been taken into consideration:

D1: J. Virol. 66, 5705-5713 (Legendre and Rommelaere, 1992)

II Novelty

D1 discloses a number of mutants in the NS1 protein of the parvovirus MVM. According to figure 1, mutants pMMBa131 and pULB3201 are still of cytotoxicity class I, showing that cytotoxicity is maintained although at a reduced level, but still regarded as being highly toxic, see page 5709 last paragraph, whereas P38 transactivation and DNA replication are strongly inhibited. pMMBa131 contains a deletion of amino acids 245-313, pULB3201 produces an NS1 with a deletion of 374 amino acids (from positions

EXAMINATION REPORT - SEPARATE SHEET

167 to 547), and these mutations are therefore located at the positions referred to in claim 5. Consequently, there can be no doubt that D1 describes NS-1 mutants and their nucleic acids with the characteristics of the NS-1 mutants and DNAs claimed in claims 1-3 and 5-9, and these claims lack novelty in view of Article 33(2) PCT.

III Inventive step

- 1. D1 is regarded as the closest prior art with respect to the question whether the claimed subject-matter involves an inventive step. The problem underlying the present application in view of D1 is the provision of further parvovirus NS1 proteins that can be used as a toxin for treating tumoral diseases.
- 2. The novel solutions to this problem provided and claimed in claim 4 of the present invention consist of four specific NS1 variants, designated as S283A, T363A, T394A and T463A. According to Table 1, these NS1 variants with the exception of T463A, are still cytotoxic, whereas P38 transactivation and DNA replication are strongly reduced. These variants can be regarded as possible candidates for use as a toxin in antitumour treatments. Hence, the subject-matter regarding NS1 variants, S283A, T363A and T394A is regarded to involve an inventive step as required by Article 33(3) PCT. Subject-matter relating to the non-toxic NS1 variant T463A is regarded not to involve an inventive step.
- 3. The antibody and kit referred to in claims 10 and 11 are characterised by the protein sequences to which these antibodies are directed. Since these antigen sequences are known from D1, and antibodies directed to known antigens are devoid of an inventive step, claims 10 and 11 also do not involve an inventive step and these claims cannot be accepted in view of Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

Claim 6 refers to DNA coding for parvovirus NS1 variants having the following phosphorylation site mutants: S283A, T363A, T394A, or T463A, wherein the DNA comprises the DNA of figure 1. The DNA of figure 1 represents the wild-type NS1. Hence, the claim is contradictory and violates the requirements of Article 6 PCT.





(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference K 2840 Wd	FOR FURTHER see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.					
International application No.	International liling date (day/month/year)	(Earliest) Priority Date (day/month/year)				
PCT/EP 00/07835	11/08/2000	13/08/1999				
Applicant						
DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.						
This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.						
This International Search Report consists of a total of sheets. X It is also accompanied by a copy of each prior art document cited in this report.						
Basis of the report						
a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.						
the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).						
 With regard to any nucleotide an was carried out on the basis of the 	d/or amino acid sequence disclosed in the inte	ternational application, the international search				
X Contained in the international application in written form.						
=	filed together with the international application in computer readable form.					
. =	furnished subsequently to this Authority in written form.					
=	furnished subsequently to this Authority in computer readble form.					
international application a	the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.					
the statement that the info furnished	the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished					
2. X Certain claims were four	nd unsearchable (See Box I).					
3. Unity of invention is lack	king (see Box II).					
4. With regard to the title,						
X the text is approved as su	the text is approved as submitted by the applicant.					
the text has been established	ned by this Authority to read as follows:					
		į.				
5. With regard to the abstract,						
the text is approved as submitted by the applicant.						
the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.						
6. The figure of the drawings to be publi	shed with the abstract is Figure No.					
as suggested by the applic		X None of the figures.				
because the applicant falls	••					
because this figure better	characterizes the invention.					

International application No. PCT/EP 00/07835

Box I Observations wher certain claims were found unsearchable (Continuation of item 1 of first sheet)
(Commission of Item 1 of first sneet)
This International Search Report has not been established in r spect of certain claims under Article 17(2)(a) for th following reasons:
Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 11 and 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the NS1 variant.
Claims Nos.: because they relate to parts of the international Application that do not comply with the prescribed requirements to such an extent that no meaningful international Search can be carried out, specifically:
Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this International application, as follows:
As all required additional search fees were timely paid by the applicant, this international Search Report covers all searchable claims.
As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest Th additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

T/EP 00/07835

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IPC 7	SIFICATION OF SUBJECT MATTER C12N15/35 C07K14/015 C07K16 A61K35/76 A61K48/00	/08 G01N33/569	C12Q1/70	
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B. FIELDS	SSEARCHED			
IPC 7	ocumentation searched (classification system followed by classific C12N C07K A61K			
	ation searched other than minimum documentation to the extent tha			
	data base consulted during the international search (name of data in ternal, STRAND, MEDLINE, BIOSIS	base and, where practical, search tel	ms used)	
	ENTS CONSIDERED TO BE RELEVANT			
Category °	Citation of document, with indication, where appropriate, of the r	elevant passages	Relevant to claim No.	
х	D LEGENDRE & J ROMMELAERE: "Terminal 1-5,7-11 regions of the NS-1 protein of the parvovirus Minute Virus of Mice are involved in cytotoxicity and promoter			
	trans inhibition" JOURNAL OF VIROLOGY, vol. 66, no. 10, October 1992 (1992-10), pages 5705-5713, XP000867510			
-	AMERICAN SOCIETY FOR MICROBIOLOGY US *mutants pMMBa131 and pULB3201; figure 1 and page 5710 first paragraph*			
		-/		
	ner documents are listed in the continuation of box C.	Patent family members ar	e listed in annex.	
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A document defining the general state of the art which is not considered to be of particular relevance inventors.				
filing da	"E" earlier document but published on or after the international filling date "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to			
citation	or other special reason (as specified)	"Y" document of particular relevance	ine document is taken alone a: the claimed invention	
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later th.	nt published prior to the international filing date but an the priority date claimed	in the art. *&* document member of the same		
Date of the a	ctual completion of the international search	Date of mailing of the internation	nal search report	
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	NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cupido, M		

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C.(Continua	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	T/EP 00/07835
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Α	LI X ET AL: "Mutation of lysine 405 to serine in the parvovirus H-1 NS1 abolishes its function for viral DNA replication, late promoter activation, and cytotoxocity" JOURNAL OF VIROLOGY	1,3,7, 9-11
	vol. 64, no. 10, October 1990 (1990-10), pages 4654-4660, XPO00867496 AMERICAN SOCIETY FOR MICROBIOLOGY US page 4656 -page 4657	
Α	J P F NÜESCH ET AL: "Sequence motifs in the replicator protein of parvovirus MVM essential for nicking and covalent attachement to the viral origin: identification of the linking tyrosine" VIROLOGY, US, ACADEMIC PRESS, ORLANDO, vol. 209, no. 1, 10 May 1995 (1995-05-10), pages 122-135, XPO02088311 ISSN: 0042-6822 page 127 -page 131	1-13
А	S F COTTMORE ET AL: "The NS1 polypeptide of the murine parvovirus MVM binds to DNA sequences containing the motif (ACCA)2-3" JOURNAL OF VIROLOGY,US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 69, no. 3, pages 1652-1660, XP002088309 ISSN: 0022-538X page 1658, left-hand column, last paragraph -right-hand column	1-13
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Parvovirus NS1 Variants

The present invention relates to parvovirus NS1 variants, DNAs coding for them and methods of producing the parvovirus NS1 variants. Furthermore, this invention concerns antibodies directed against the parvovirus NS1 variants as well as the use of the DNAs and the parvovirus NS1 variants.

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Parvovirus designates a genus of the virus family Parvoviridae. The parvovirus genus comprises a number of small, icosaedric viruses that can replicate in the absence of a helper virus. Parvovirus contains a single-stranded DNA having a length of about 5.000 bp. At the 3' and 5' ends of the DNA there is one palindromic sequence each. The DNA codes for two capsid proteins, VP1 and VP2, as well as for two regulatory non-structure proteins, NS-1 and NS-2. The latter proteins are phosphorylated and show nuclear or both cytoplasmic and nuclear localization, respectively. NS1 is necessary for viral DNA replication and participates in the regulation of viral gene expression. Particularly, NS1 transactivates the promoter P38 and exhibits DNA-binding, helicase and DNA-nicking activities. Furthermore, NS1 induces cytotoxic and/or cytostatic stress in sensitive host cells.

Parvoviruses are usually well-tolerated by populations of their natural host, in which they persist without apparent pathological signs. This is due to both the protection of foetuses and neonates by maternal immunity, and the striking restriction of parvovirus replication to a narrow range of target proliferating tissues in adult animals. This host tolerance concerns especially rodent parvoviruses, for example the minute virus of mice (MVM) and H-1 virus in their respective natural hosts, namely mice and rats. In addition, humans can be infected with the latter viruses, without any evidence of associated deleterious effects from existing

epidemiological studies and clinical trials. On the other hand, it is known that certain parvoviruses, and especially rodent parvoviruses, are both oncotropic, i.e. accumulate preferentially in neoplastic versus normal tissues, and oncosuppressive, i.e. have a tumor-suppressive effect towards tumor cells, in various animal models. At least part of the oncosuppressive effect is thought to be due to a direct oncolytic action mediated by NS1. This oncosuppressive effect was also demonstrated against human tumor cells transplanted in recipient animals.

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It is considered to use parvoviruses for therapeutic purposes. On the one hand, it seems to be of interest to use parvoviruses as vectors for therapeutic genes, i.e. for introducing such genes into the genome of cells. On the other hand, it is considered to use NS1 of parvoviruses as a toxin for treating tumoral diseases. However, initial experiments showed unsatisfactory results.

Therefore, it is the object of the present invention to provide a product by which parvoviruses and NS1 thereof, respectively, can be used for the above purposes.

According to the invention this is achieved by the subject matters defined in the claims.

The present invention is based on the applicant's findings that it is possible to interfere with the activities of parvovirus NS1 so as to shift the equilibrium existing between the DNA replication and transcription activities (a) and the cytotoxicity activity (b). In particular, he produced parvovirus NS1 variants in which the DNA replication and transcription activities (a) are reduced and eliminated, respectively, whereas the cytotoxicity activity (b) is maintained or raised. Moreover, he produced parvovirus NS1 variants in which the cytotoxicity activity (b) is reduced and eliminated, respectively, whereas the DNA replication and transcription activities (a) are maintained or raised.

Examples of such parvovirus NS1 variants are indicated in Table 1 and figure 1. In addition, the applicant recognized that the above parvovirus NS1 variants and expression vectors coding for them, particularly parvoviruses, respectively, are suitable for the application purposes.

According to the invention, the applicant's findings are used to provide a parvovirus NS1 variant in which the equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b) is shifted.

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The expression "parvovirus" comprises any parvovirus, particularly a rodent parvovirus, such as minute virus of mice (MVM) and H-1 virus.

The expression "the equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b) is shifted refers to the fact that in a parvovirus NS1 variant according to the invention such an equilibrium is shifted as compared to the parvovirus NS1 wild-type. In particular, the equilibrium can be shifted to the effect that the DNA replication and transcription activities (a) are reduced and eliminated, respectively, whereas the cytotoxicity activity (b) is maintained or raised. The cytotoxicity activity (b) can also be reduced and eliminated, respectively, whereas the DNA replication and transcription activities (a) are maintained or raised. Such an equilibrium can be determined by various methods. As regards the determination of the DNA replication activity, reference is made e.g. to methods described in Legendre and Rommelaere, 1992, J. Virol. 66, 5705; Cotmore et al., 1992, Virology 190, 365; Cotmore et al., 1993, J. Virol. 67, 1579, Cotmore and Tattersall, 1994, Embo. J. 13, 4145. As to the determination of the transcription activity reference is made to methods described e.g. in Rhode and Richards, 1987, J. Virol. 61, 2807. Regarding the determination of the cytotoxicity activity reference is made to the below examples.

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According to the invention parvovirus NS1 variants are preferred in which the shift of equilibrium is achieved by mutation of one or several phosphorylation sites. Particularly preferred are parvovirus NS1 variants which have a mutation at one or several of the phosphorylation sites 283, 363, 394 and 463. Even more preferred are the parvovirus NS1 variants S283A, T363A, T394A and T463A, which are indicated in Table 1 and figure 1. In S283A, a serine is exchanged by an alanine at position 283, in T364A a threonine is exchanged by alanine at position 363, in T394A a threonine is exchanged by alanine at position 394 and in T 463A a threonine is exchanged by alanine at position 394 and in T 463A a threonine is exchanged by alanine at position 463.

A further subject matter of the present invention relates to a nucleic acid, particularly a DNA, which codes for an above parvovirus NS1 variant. Such a DNA comprises preferably:

- (a) the DNA of fig. 1.1, 1.2, 1.3 and 1.4, respectively
- (b) a DNA hybridizing with the DNA from (a), said DNA 20 comprising the mutated phosphorylation site of the DNA from (a), or
 - (c) a DNA related to the DNA from (a) or (b) via the degenerated genetic code.
- 25 The DNA of (a) was deposited with DSMZ (Deutsche Sammlung von Mikroorganismen and Zellkulturen) on Aug. 11, 1999, i.e. fig. 1.1 as Escherichia coli pRSV-NS:S283A under DSM 12994, fig. 1.2 as Escherichia coli pRSV-NS:T363A under DSM 12995, fig. 1.3. as Escherichia coli pRSV-NS:T394A under DSM 12996 and fig. 1.4 as Escherichia coli pRSV-NS:T463A under DSM 12997.

The expression "hybridizing DNA" refers to a DNA which hybridizes with a DNA from (a) under normal conditions, particularly at 20(C below the melting point of the DNA. In this connection, the expression "hybridizing" refers to conventional hybridization conditions, preferably to hybridization conditions where 5xSSPE, 1 % SDS, 1xDenhardt's solution are used as solution and the hybridization

temperatures are between 35(C and 70(C, preferably 65(C. The hybridization is followed by a wash step first carried out with 2xssc, 1 % SDS and then with 0.2xssc at temperatures between 35(C and 70(C, preferably at 65(C. Furthermore, reference is made to sambrook et al., Molecular Cloming: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory

Press, cold Spring Harbor NY (1989).

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A DNA according to the invention can be present in a vector and expression vector, respectively. A person skilled in the art is familiar with examples thereof. In the case of an expression vector for E. coli these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b, T7 based expression vectors and pQE-8. For the expression in yeast, e.g. pY100 and Ycpad1 have to be mentioned while e.g. pKCR, pEFBOS, cDM8, pMSCND, and pCEV4 have to be indicated for the expression in animal cells. The baculovirus expression vector pAcSGHisNT-A is especially suitable for the expression in insect cells.

In a preferred embodiment, the vector containing the DNA according to the invention is a virus, e.g. an adenovirus, vaccinia virus, an AAV virus or a parvovirus, such as MVM or H-1, a parvovirus being preferred. The vector may also be a retrovirus, such as MoMULV, MoMuLV, HaMuSV, MuMTV, RSV or GaLV.

For constructing expression vectors which contain the DNA according to the invention, it is possible to use general methods known in the art. These methods include e.g. in vitro recombination techniques, synthetic methods and in vivo recombination methods as described in Sambrook et al., supra, for example.

Furthermore, the present invention relates to host cells which contain the above described vectors. These host cells include bacteria, yeast, insect and animal cells, preferably mammalian cells. The E. coli strains HB101, DH1, x1776, JM101, JM109, BL21, XL1Blue and SG 13009, the yeast strain

Saccharomyces cerevisiae and the animal cells L, A9, 3T3, FM3A, CHO, COS, Vero, HeLa and the insect cells sf9 are preferred. Methods of transforming these host cells, of phenotypically selecting transformants and of expressing the DNA according to the invention by using the above described vectors are known in the art.

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Moreover, the present invention relates to antibodies which specifically recognize an above describe parvovirus NS1 variant, i.e. the region of the parvovirus NS1 variant where the mutation responsible for the shifted equilibrium. particularly a mutated phosphorylation site, is located. The antibodies can be monoclonal, polyclonal or synthetic antibodies or fragments thereof, e.g. Fab, Fv or scFV fragments. Preferably monoclonal antibodies are concerned. For the production it is favorable to immunize animals particularly rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above parvovirus NS1 variant or with fragments thereof. Further boosters of the animals can be effected with the same parvovirus NS1 variant or with fragments thereof. The polyclonal antibody can then be obtained from the animal serum and egg yolk, respectively. The monoclonal antibody can be obtained according to standard methods, reference being made particularly to the method by K+hler and Milstein (Nature 256 (1975), 495) and Galfrú (Meth. Enzymol. 73 (1981), 3). In this case, mouse myeloma cells are fused with spleen cells originating from the immunized animals. Antibodies according to the invention can be used in many ways, e.g. for the immunoprecipitation of the above described parvovirus NS1 variants or for the isolation thereof. The antibodies can be bound in immunoassays in liquid phase or to a solid carrier. In this connection. antibodies can be labeled in various ways. The person skilled in the art is familiar with suitable markers and labeling methods. Examples of immunoassays are ELISA and RIA.

The present invention provides parvovirus NS1 variants in which the equilibrium between the DNA replication and

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transcription activities (a) and the cytotoxicity activity (b) is shifted. In particular, parvovirus NS1 variants are provided which have a reduced or no cytotoxicity activity, whereas the DNA replication and transcription activities are wainiained or increased. Parveying NSI veriente ese else provided in which the DNA replication and transcription activities are reduced and eliminated, respectively, whereas the cytotoxicity activity is maintained or raised. Thus, the present invention provides products which are suitable for therapeutic purposes. In particular, expression vectors according to the invention, e.g. parvoviruses, can be used for gene-therapeutic measures. Moreover, parvoviruses NS1 variants according to the invention are suitable as toxins, e.g. for treating tumoral diseases.

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Therefore, a kit is also provided for the application of the present invention. This kit comprises the following:

- (a) a parvovirus NS1 variant according to the invention,
- (b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,
 - (c) an antibody according to the invention, as well as
 - (d) conventional auxiliary agents, such as solvents, buffers. carriers, markers and controls.

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Of component (a) to (d) one or more representatives can be present each.

Brief description of the drawings

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Fig. 1 shows the DNA and amino acid sequences of parvovirus NS1 variants according to the invention (fig. 1.1, 1.2, 1.3 and 1.4) as compared to a parvovirus NS1 wild-type. In this connection, the mutated sites in the parvovirus NS1 variants according to the invention are labeled each.

The present invention is explained by the examples.

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Example 1: Preparation and purification of NS1 variants according to the invention

The DNA of the NS1 variant S283A according to the invention was provided as an EcoRV to BstEII fragment obtained by chimeric PCR using two mutagenic primers. This fragment was then inserted into the corresponding cleaved expression vector pTHisNS1 (Nuesch et al., Virology 209, (1995), 122) to obtain pTHis NS1:S283A. Such a vector codes for a fusion protein comprising 6 histidine residues (N terminus partner) and S283A of Fig. 1 (C terminus partner). For expression and purification of S283A the NS1 gene under control of the bacteriophage T7 promoter was transferred into vaccinia virus and expressed in eucaryotic cells by double infection together with vTF7-3 (a vaccinia virus expressing the bacteriophage T7 DNA polymerase). 18 hrs post infection cells were harvested and nuclear extracts prepared. The histidine tagged S283A was then purified by affinity chromatography on Ni-NTA agarose and analyzed by 10 % SDS-PAGE (Nuesch et al., supra).

It showed that a parvovirus NS1 variant according to the invention can be prepared in highly pure form.

The NS1 variants T363A, T394A, and T463A were also produced and purified in the same way.

Example 2: Preparation and detection of an antibody according to the invention

Tubes were coated with purified NS1 variants prepared as in example 1 and monoclonal antibodies (e.g. scFv) specifically binding to S283A were isolated from human synthetic VH+VL scFv phage library (Griffith et al., EMBO J., 13, (1994), 3245) according to standard panning protocols after >5 isolation and amplification procedures. The variable region of the isolated scFv harbored in the phagemid were sequenced to identify NS1

variant interacting partner proteins harboring such binding motifs from comparison with known genes in the gene bank.

It showed that monoclonal antibodies according to the invention can be isolated.

In addition, the NS1 variants were used for immunization of animals in order to obtain poly- or monoclonal antibodies.

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Example 3: Characterization of the parvovirus NS1 variants S283A, T363A, T394A and T463A according to the invention

The characterization of the parvovirus NS1 variants comprised the determination of the DNA replication, transcription, cytotoxicity, DNA binding, nicking and helicase activities. Known methods were used for this purpose (cf. description, supra). As regards the determination of the helicase activity reference is made to Stahl et al. 1986, EMBO J. 5, 1999. As to the determination of the nicking activity reference is made to Christensen et al., 1997, J. Virol. 71, 1405 and Nuesch et al., 1995, supra. Regarding the determination of the DNA binding reference is made to Cotmore et al. 1995, J. Virol. 69, 1652. As far as the determination of the cytotoxicity activity is concerned, the following steps were carried out: NS1 variants were transferred into an expression vector containing the NS1 gene under the control of the parvovirus MVMP4 promoter (genuine promoter driving the non-structural genes of MVM), and the green fluorescent protein (EGFP) under control of an additional promoter. These constructs were then transfected into A9 cells using lipofectamine (GibcoBRL) according to the manufacturer's instruction and the impact of the NS1 variant on the viability of the cells tested in time course experiments. Transfected cells were identified by fluorescence of the EGFP. Toxic effects were determined in comparison to wild type NS1 or a vector containing no NS1 gene as a function of time as well as a measure of cytopathic

changes on the cell morphology.

The data indicated in Table 1 were obtained:

mahle 1

S283A T363A T394A T463A wt P38-TA ++++ ++++ ACCA + ++++ ++ ++ Nick-1 + +++ +++ Nick-2 +++ ++++ ++++ Nick-3 ++ ++++ Heli ++ _ (+)++++ ++++ Rep + + ++++ +++ Cvto +++++ +++ (+)

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Example 4: NS1 variants' expression after transduction using recombinant viral vectors

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NS1 expression cassettes containing the NS1 variants according to the invention under control of the parvoviral P4 promoter and a 3'untranslated region from parvovirus MVM to ensure stability and translation of the gene product, were transferred either in a parvovirus genome background as exemplified in example 3, or a heterologous viral genome background, such as vaccinia virus (example 1) or adenovirus. Promoter and terminator regions were exchanged according to the requirements. The nucleic acids containing the NS1 variants were then packaged either in vivo (after transient transfection into eucaryotic cells) or in vitro and the packaged transducing particles were isolated. These transducing units containing NS1 variants were used either for studies concerning gene regulation in tissue culture or animals, but also as therapeutic agents either alone or in combination with other agents (such as cytokines) in gene and cancer therapy approaches.

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Claims.

- A parvovirus NS1 variant having a shifted equilibrium
 between the DNA replication and transcription activities
 (a) and the cytotoxicity activity (b).
 - The parvovirus NS1 variant according to claim 1, wherein the activities (a) are reduced and eliminated, respectively, and activity (b) is maintained or increased.
 - The parvovirus NS1 variant according to claim 1, wherein activity (b) is reduced and eliminated, respectively, and the activities (a) are maintained or increased.
 - The parvovirus NS1 variant according to any one of claims 1 to 3, wherein one or several phosphorylation sites are mutated.
 - The parvovirus NS1 variant according to claim 4, wherein the mutations are located at sites 283, 363, 394 and/or 463.
 - The parvovirus NS1 variant according to claim 4 or 5, namely the NS1 variants S283A, T363A, T394A, and T463A.
 - A DNA, coding for the parvovirus NS1 variant according to any one of claims 1 to 6.
 - 8. The DNA according to claim 7, wherein the DNA comprises:
 - (a) the DNA of figure 1,
 - (b) a DNA hybridizing with the DNA from (a), said comprising the mutated phosphorylation site of the DNA from (a), or
 - (c) a DNA related to the DNA from (a) or (b) via the $\mbox{degenerated genetic code}.$

 An expression vector, comprising the DNA according to claim 7 or 8.

- 10. A transformant, containing the expression vector
- 11. A method of producing the parvovirus NS1 variant according to any one of claims 1 to 6, comprising the culturing of the transformant according to claim 10 under suitable conditions.
- An antibody, directed against the parvovirus NS1 variant according to any one of claims 1 to 6.
- 15 13. Kit comprising:

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- (a) a parvovirus NS1 variant according to the invention,
- (b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,
- (c) an antibody according to the invention, as well as
- (d) conventional auxiliary agents, such as solvents, buffers, carriers, markers and controls,

- 14. Use of the parvovirus NS1 variant according to any one of claims 1 to 6 as a toxin for treating tumoral diseases.
- 15. Use of the DNA according to claim 9 as a vector for gene 30 therapy.

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Fig. 1

Wild-type NS1

261				+-							+			-+-				+		AAAA TITT	320
	М	A	G	N	A	Y	s	D	Ε	v	L	G	A	T	N	W	L	ĸ	Ε	K	-
321				-+-						LAA.	AT	TTT	ACT	TTI	ACA	AGI	TG	CT	ACC	TITI	380
381	GA	TAT	cgg	ATC	GA.	TAC	TTA	CAJ	AAA		+			-+-						K ACAA TGTT	440
	D	I	-	W		_	_		K	_		Q		_	E	L	K	s	ь	Q	-
441				-+-			+				+			-+-			+			GGAT + CCTA	500
	R	G	A	E	T	T	W	D	Q	s	E	D	M	Ξ	W	Ε	T	T	v	D	-
501				-+-			+				÷			-÷-			+			TGAA + ACTT	560
	Ξ	M	T	K	K	Q	v	F	I	F	D	s	L	٧	ĸ	ĸ	С	L	F	E	-
561				-+-	-		+				+			-+-	- - -		+			ATGG + TACC	620
	V	L	N	T	K	И	I	F	P	G	D	V	N	W	F	V	Q	H	Ε	W	-
621				-+-			+				+			-+-			+			AGCT CGA	680
	G	ĸ	D	Q	G	W	H	С	H	٧	L	Ι	G	G	K	D	F	s	Q	A	-
681				-+-			+				÷						+			AGCC TCGG	740
	Q	G	ĸ	W	W	R	R	Q	L	N	v	Υ	W	s	R	W	L	v	T	A	-
741	TG:	raa'	IGT	GCA		AAC										AAT.	AGC	AGA	AGA	TAAT	800

2/6 Fig. 1 (Fortsetzung I)

	AC	TTA	ACA	CGT	TGA	TTG	TGC	TCC	ACT	TTC	TTA	ATI	'TGA	TTC	TCI	TTA	TCC	TCT	TCI	GTTA	
	С	N	V	Q	L	T	Þ	A	E	R	1	ĸ	L	R	E	I	A	E	D	N	-
	GA	.GTG	GGI	TAC	TCI	ACT	TAC	TT	ATA	.GC	TAA	.GCA	AAC	CAA	AAA					GTGT	
	CT	CAC	CCA	ATG	AGA	TGA	ATC	:AA	AT	CGI	ATI	CGI	TIC	GTI	TTT					CACA	
	Б	W	V	T	L	L	T	Y	K	н	ĸ	Q	T	к	к	D	Y	T	ĸ	С	-
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861																				ATCA	
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921																				TTTA	
921																				TAAA	980
	P	P	R	D	G	G	Y	F	L	s	s	D	s	G	W	ĸ	T	N	F	L	-
001																				AACG	1040
301																					1040
	ĸ	E	G	E	R	Н	L	v	s	K	L	Y	T	D	D	M	R	P	E	T	-
1041	TTTCTTCCGCTCGCGGTAGATCACTCGTTTGATATGTGACTACTGTACGCCGGTCTTTGC K E G E R H L V S K L Y T D D M R P E T GTTGAAACCACAGTAACCACTGCGGAGAAACTAAGCGCGGCAGAATTCAAACTAAAAAA CAACTTTGGTGTCATTGGTGACGCGTCCTTTGATTCGCGCCGTCTTAAGTTTGATTTTTT V E T T V T T A Q E T K R G R I Q T K K																				
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	E	V	S	I	ĸ	T	T	L	ĸ	E	L	V	Н	K	R	V	T	s	P	Ē	-
1161																				AGAA	1220
	CT	GAC	CTA	CTA	CTA	CGT	CGG	TCT	GTC	AAT	GTA	ACT	ΓTΑ	CTA	CCG	AGT	TGG	rcc	ACC	CTT	
	D	W	М	М	М	Q	P	D	s	Y	I	E	M	М	A	Q	P	G	G	E	-
1221																				AGCA	1280
	TT	GGA	CGA	CTT:	TTT.	ATG	CGA	TCT	CTA	AAC	ATG'	TGA'	TTG	AGA:	rcg	GTC	TTG	JTT.	rTG'	rcgt	
							L														-
1281	TT	TGA	CTT.	AAT:	TTT.	AGA	AAA.	AGC	TGA	AAC	CAG	CAA	ACT	AAC	CAA	CTT	TTC	ACT	CC.	IGAC	1340
																				ACTG	
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1341				-+-			+				+			-+-			+			IGCT	1400
	TG:	TTC	TTG	GAC	JTC.	TTA	AAA	ACG	AAA	AGT	ACC	GAC	CTT	GAT	ACA	ATT	TCA	AAC	GT.	ACGA	

3/6 Fig. 1 (Fortsetzung II)

140	· - 1			GIG	111	IAA	ACA	GAC	AAG	GAG	GCA	AAA	LGA,	ATA	CI	STT	TTA	TT.	TCA	TGC	JACC:	A.
140		וממי	7007	+	777	7 7		+			-+-							-+-				+ 1460
		·	1002	ساءر	MAM	WII	161	CIG	TTC	CTC	CGT	TTT	CT	TA.	rga	CAA.	AAT	AA	\GT	'ACC	TGG	r
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146	٠, ۲	3CC2	AGCA	CAG	GCA.	AAT	CTA'	TTA'	TTG	CAC	AAG	CCA	TAC	CAC	AAC	CAC	GTT	GG	AA	TGI	TGG:	2
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1821				-+-			+-							-+-		-		+			+	1880
	-	ACI	GAA	ACC!	AAA	CAA	CTC	31-1-1	117	CT	CAC	CGG	GTA	CTA	AAC	ACC	AA	CCA	AC	CAT	TTC	
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1881	AA	TGG	TTA	CCA	ATCI	racc	ATO	GCA	AGC	TAC	TG:	rgc:	TAA	ATG	GGG	CAZ	AG	TTC	CI	GAT		
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2001				+			-+-			+				-+-							+	2060

4/6 Fig. 1 (Fortsetzung III)

SPFTTPKSTPLSQNYALTPL

ASDLEDLALEPWSTPNTPVA.

GTAETQNTGEAGSKACQDGQ -

LSPTWSEIEEDLRACFGAEP -

TTGAAGAAGACTTCAGCGAGCCGCTGAACTTGGACTAA
2241 ------ 2279

AACTTCTTTCTGAAGTCGCTCGGCGACTTGAACCTGATT

LKKDFSEPLNLD*

5/6

Fig. 1.1

1100 - 261 Wildtype-NS1-Sequence

GAAGTITCTATTAAAACTACACTTAAAGAGCTGGTGCATAAAAGAGTAACCTCACCAGAG

1101
CTTCAAAGAGATAATTTTGATGTGAATTTTCTCGACCACGTATTTTCTCATTGGAGTGGTCTC

E V S I K T T L K E L V H K R V T S P E

1161 - 2279 Wildtype-NS1-Sequence

Fig. 1.2

1340 - 261 Wildtype-NS1-Sequence

TRTCRIFAFHGWNYVKVCHA-→A T3C3A

1401 - 2279 Wildtype-NS1-Sequence

6/6

Fig. 1.3

1400 - 261 Wildtype-NS1-Sequence

1461 - 2279 Wildtype-NS1-Sequence

Fig. 1.4

1640 - 261 Wildtype-NS1-Sequence

GGTCARRCTATTCGCATTGATCAAAAAGGAAAAGGCAAACAGATTGAACCAACACA

1641

CCAGTTGATAGCGTAACTAGTTTTTCCTTTTCCGTCGTTTGTCTAACTTGGTTGTGGT

G Q T R I D Q K G K G S K Q I E P T P - A T 463A

1701 - 2279 Wildtype-NS1-Sequence

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SEQUENCE LISTING

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<pre><211> 2019 <212> DNA <213> Wildtype Parvovirus NS1 <220> <221> CDS <222> (1)(2016) <400> 1 atg gct gga aat gct tac tct gat gaa gtt ttg gga gca acc aac tgg</pre>														
Met Ala Gly Asn Ala Tyr Ser Asp Glu Va	tt ttg gga gca acc aac tgg 48 al Leu Gly Ala Thr Asn Trp 10 15													
tta aag gaa aaa agt aac cag gaa gtg ti Leu Lys Glu Lys Ser Asn Gln Glu Val Pl 20 25														
gaa aat gtt caa ctg aat gga aaa gat at Glu Asn Val Gln Leu Asn Gly Lys Asp I 35 40														
aaa gag ctg cag gag gac gag ctg aaa to Lys Glu Leu Gln Glu Asp Glu Leu Lys So 50 55														
act act tgg gac caa agc gag gac atg ga Thr Thr Trp Asp Gln Ser Glu Asp Met G 65 70	aa tgg gaa acc aca gtg gat 240 lu Trp Glu Thr Thr Val Asp 75 80													
gaa atg acc aaa aag caa gta ttc att tt Glu Met Thr Lys Lys Gln Val Phe Ile Ph 85	tt gat tct ttg gtt aaa aaa 288 he Asp Ser Leu Val Lys Lys 90 95													
tgt tta ttt gaa gtg ctt aac aca aag aa Cys Leu Phe Glu Val Leu Asn Thr Lys As 100 105														
aat tgg ttt gtg caa cat gaa tgg gga aa Asn Trp Phe Val Gln His Glu Trp Gly Ly 115 120	aa gac caa ggc tgg cac tgc 384 ys Asp Gln Gly Trp His Cys 125													
cat gta cta att gga gga aag gac ttt ag His Val Leu Ile Gly Gly Lys Asp Phe Se 130														

tgt aat gtg caa cta aca cca gct gaa aga att aaa cta aga gaa ata Cys Asn Val Glu Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile gca gaa gac aat gag tgg gtt act cta ctt act tat aag cat aag caa Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln 180 acc aaa aaa gac tat acc aag tgt gtt ctt ttt gga aac atg att gct Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala 205 tac tat ttt tta act aaa aag aaa ata agc act agt cca cca aga gac Tyr Tyr Tyr Phe Leu Thr Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp 210 gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act act ttt ta 220 gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act act ttt ta 220 gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act act ttt ta 220 gga ggc tat troug agc agt gac cat caa gta gac act act gtr Tyr Dyr Phe Leu Ser Ser Asp Ser Gly Typ Lys Thr Asn Phe Leu 225 gga ggc tat troug acc act cta gtg acc act Lyg Lys Leu Tyr Thr Asp Asp Met 225 cgg cca gaa acg gtt gaa acc aca gta acc act act gcg cag gaa act aac act gar gac act agr pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys 260 cgg cca gaa acg gtt gaa acc aca gta acc act gcg cag gaa act aag 816 cgg cag aga att caa act aaa aag aga gtt tct att aaa act aca ctt Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu 275 aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg 275 aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg 275 aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg 275 aaa gag ctg gtg cat acat tt gaa acc acc ta acc cac gag gac tgg gaa acc acc acc gac aga acc act act gac acc acc acc acc acc acc acc acc acc	tgg Trp 145	aga Arg	agg Arg	caa Gln	cta Leu	aat Asn 150	gtt Val	tac Tyr	tgg Trp	agc Ser	aga Arg 155	tgg Trp	ttg Leu	gta Val	aca Thr	gcc Ala 160		480
Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln 180 acc aaa aaa gac tat acc aag tgt gtt ctt ttt gga aac atg att gct Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala 200 tac tat ttt tta act aaa aag aaa ata agc act agt cca cca aga gac Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp 210 gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act aact tt tta Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu 225 aaa gaa ggc gag cgc cat cta gtg agc aaa cta tac act gat gac atg Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met 226 cgg cca gaa acg gtt gaa acc aca gta acc act gcg cag gaa act aag Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys 260 cgc ggc aga att caa act aaa aaa gaa gtt tct att aaa act aca ctt Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu 275 aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg Arg Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met 280 atg cag cca gaa agt ta att gaa atg atg gct caa cca ggt gag gaa Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg Asn Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Glu Thr Leu Ala Arg Asn Pro Asp Ser Leu Pro Asp Thr Asp Thr Asp Thr Ser Lys 340 cca aaa aca gca ctt cac ctt gac aca aca aca gca cac aca aca gca aca Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg Asn Pro Asp Ser Leu Pro Asp Thr Asp Thr Cys Arg Ile Phe Ala 355 ctt cat ggc tgg aca tat gca ta att tac aca aca gca cac aca aca gca aca 1008 cta acc aca ttt tca ctg cct gac aca aca aca gca tta ttt gct 260 cta acc aca cttt tca ctg cct gac aca aca aca ctga cat gca ctg 370 cta acc aca cttt tca ctg ct gac aca aca aca ctga cat gct 370 cta acc aca ttt tca ctg ct gac aca aca aca ctga cat gct 370 370 cta acc aca gac aca gac act att tta aca aca aca ctga cac gca 370 370 cta aca gac can gac act tat ctt ta aca gct ttt ttt ttt cat ggc 38	tgt Cys	aat Asn	gtg Val	caa Gln	Leu	aca Thr	cca Pro	gct Ala	gaa Glu	Arg	att Ile	aaa Lys	cta Leu	aga Arg	Glu	ata Ile		528
This Lys Lys Asp Tyr This Lys Cys Val Leu Phe Gly Ash Met Ile Ala 205 tac tat titt the act ase asg as at asg cat agt cca cca age gac 72 210 gga ggc tat tit ctt agc agt gas tot ggc tgg ase act agt cca cca age gac 672 gga ggc tat tit ctt agc agt gac tct ggc tgg ase act act tit ta 225 230 ase gas ggc gag cgc cat cta gtg agc ase cta tac act gat gac atg Lys Glu Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Ash Phe Leu 225 230 ase gas agg gag cgc cat cta gtg agc ase cta tac act gat gac atg Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met 255 cgg cca gas acg gtt gas acc aca gts acc act gcg cag gas act asg Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Glu Thr Lys 255 cgc ggc age att cas act ase ase gas gtt tct att ase act aca ctt Arg Gly Arg Ile Glu Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu 286 ase gag ctg gtg cat ase ase age gts acc tca cca gag gac tgg atg atg Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met 290 atg cag cca gac agt tac att gas atg atg gct cas cca ggt ggs gg gas ge gas act asg Met Glu Pro Asp Ser Tyr Ile Glu Met Met Ala Glu Pro Gly Gly Glu 310 sac ctg ctg ase ast cg cta gag att tat att as act act cta gcc age Ash Leu Leu Lys Ash Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 310 acc cas aca gca ctt ta gct act att ta gas aca gct gas acc aca acc aca acc aca acc aca acc ac				Asn					Leu					His				576
Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp 210 gga ggc tat ttt ctt agc agt gac tct ggc tgg aaa act aac ttt tta CJY 230 aaa gaa ggc gag cgc cat cta gtg agc aaa cta tac act gat gac atg Lys Glu Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu 230 aaa gaa ggc gag cgc cat cta gtg agc aaa cta tac act gat gac atg Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met 255 cgg cca gaa acg gtt gaa acc aca gta acc act gcg cag gaa act aag Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Glu Glu Thr Lys 255 cgc ggc aga att caa act aaa aaa gaa gtt tct att aaa act aca ctt Arg Gly Arg Ile Glu Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu 285 aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met 290 atg cag cca gac agt tac att gaa atg atg gct caa cca ggt gga gaa gac tgg atg atg 191 acc ctg ctg aaa aat acg cta gag att tct act act act cta gcc aga Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 310 acc ctg ctg aaa act acg cta gat att tta gaa aac gct gaa acc aca gac aca Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 310 cta acc act ttt cac ctg cct gac aca aga acc tgc aga att ttt gct Leu Thr Asn Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys 340 cta acc aca cttt tca ctg cct gac aca aga acc tgc aga att ttt gct Leu Thr Asn Phe Ser Leu Pro Asp Thr Asp Thr Cys Arg Ile Phe Ala 360 cta acc aca cttt tca ctg cct gac aca aga acc tgc aga att ttt gct Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala 370 cta acc aca gac aca gag acc aca gac aca acc aca gca cac gcc aca gac acc ac	acc Thr	aaa Lys	Lys	gac Asp	tat Tyr	acc Thr	aag Lys	Cys	gtt Val	ctt Leu	ttt Phe	gga Gly	Asn	atg Met	att Ile	gct Ala		624
Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu 230 235 240 240 235 240 235 240 235 240 235 240 235 255 255 255 255 255 255 255 255 255		Tyr					Lys					Ser						672
Lys Glu Gly Glu Arg His Leu Val Ser Lys Lu Tyr Thr Asp Asp Met 2256 cgg cca gaa acg gtt gaa acc aca gta acc act gcg cag gaa act aaag Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Glu Glu Thr Lys 265 cgg cag gaa act aaag acc aca gta acc act gcg cag gaa act aaag Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Glu Glu Thr Lys 265 cgg cag gac act aca act aca cac cgc ggc aga att caa act aca act aca gag gtt tct att aaa act aca ctt arg Gly Arg Ile Glu Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu 285 cac aca gag gac tgg gtg cat aca aca aga gta acc tca cca gag gac tgg atg atg Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met 290 cac gac aga agt tac att gaa atg atg gtg cat acc aca ggt gga gac met Glu Pro Asp Ser Tyr Ile Glu Met Met Ala Glu Pro Gly Gly Glu 200 cac ctg ctg aca act act gca gag att tct act gca gag Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 315 cac aca aca gca ctt gac ta act tta gaa aca gca ctg cac aca aca gca aca gca cac aca gac aca gca cac aca aca	Gly					Ser					Trp					Leu		720
Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys 260 270 cgc ggc aga att caa act aaa aaa gaa gtt tct att aaa act aca ctt Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu 275 aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met 300 atg cag cca gac agt tac att gaa atg gt ccaa cca gag gac tgg aga gaa Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Gly Glu Glu 310 acc ctg ctg aaa aat acg cta gag att tgt aca cta act cta gc aga Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 325 acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc ag aca aga acc aga caa att tta gac aca aga acc ag aca aga acc aga acc aga acc aga acc acc	aaa Lys	gaa Glu	ggc Gly	gag Glu	Arg	cat His	cta Leu	gtg Val	agc Ser	Lys	cta Leu	tac Tyr	act Thr	gat Asp	Asp	atg Met		768
Arg diy Arg Ile din Thr Lys Lys div Val Ser Ile Lys Thr Thr Leu 275 aaa gag ctg gtg cat aaa aga gta acc tca cca gag gac tgg atg atg 290 atg Cag cca gac agt tac att gaa atg atg gct caa cca ggt gga gga aga atg atg 290 atg Cag cca gac agt tac att gaa atg gct caa cca ggt gga gaa Met Gin Pro Asp Ser Tyr Ile Giu Met Met Ala Gin Pro Giy Giy Giu 320 acc ctg ctg aaa aat acg cta gag att tgt aca cta act cta gcc aga Asn Leu Leu Lys Asn Thr Leu Giu Ile Cys Thr Leu Thr Leu Ala Arg 325 acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc ag aca aga acc agc aaa ach 345 acc aac aca gca ttt gac tta att tta gau aaa gct gaa acc agc aaa Thr Lys Thr Ala Phe Asp Leu Ile Leu Giu Lys Ala Giu Thr Ser Lys 346 cta acc aca ctt tca ctg cct gaa aca aga acc tgc aga att tt gct Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala 355 ttt cat ggc tgg aac tat gtt aaa gtt tgc cat gct att tgc tgt gtt Phe His Giy Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val 370 acc aga caa gga ggc aaa aga aat act gtt tta ttt cat gga cca Leu Asn Arg Gin Ciy Giy Lys Arg Asn Thr Val Leu Phe His Giy Pro 390 gcc agc aca gga gag aaa tct att att gca caa gca ata gca caa gag act gct agc act agc gct gtt Ala Ser Thr Giy Lys Ser Ile Ile Ala Gin Ala Ile Ala Gin Ala Val	cgg Arg	cca Pro	gaa Glu	Thr	gtt Val	gaa Glu	acc Thr	aca Thr	Val	acc Thr	act Thr	gcg Ala	cag Gln	Glu	act Thr	aag Lys		816
Lys Giu Leu Val His Lys Arg Val Thr Ser Pro Giu Asp Trp Met Met 300 atg cag cca gac agt tac att gaa atg gct caa cca ggt gga gaa Met Gin Pro Asp Ser Tyr Ile Giu Met Met Ala Gin Pro Gly Gly Glu 310 315 acc ctg ctg aaa sat acg cta gag att tgt aca cta act cta gcc aga Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 325 acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc agc aaa aca aca aga aca aca aca			Arg					Lys					Lys					864
Met Gin Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Gly Glu 305 acc ctg ctg aaa aat acg cta gag att tgt aca cta act cta gcc aga Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 325 acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc agc aaa Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys 340 cta acc aac ttt tca ctg cct gac aca aga acc tgc aga att ttt gct Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala 355 ttt cat ggc tgg aac tat gtt aaa gtt tgc cat gct att tgc tgt gtt Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val 370 tta aac aga caa gga ggc aaa aga ata act gtt tta ttt cat gga cca Leu Asn Arg Gln Cly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 gcc aga caa ggc aaa tct att att gac caa gca aga ggt gc agc aca ggc aga ttat gac aca aga gac gtt Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val	aaa Lys	Glu	ctg Leu	gtg Val	cat His	aaa Lys	Arg	gta Val	acc Thr	tca Ser	cca Pro	Glu	gac Asp	tgg Trp	atg Met	atg Met		912
Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 325 acc aaa aca gca ttt gac tta att tta gaa aaa gct gaa acc agc aaa Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys 340 cta acc aac ttt tca ctg cct gac aca aga acc tgc aga att ttt gct Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala 355 ttt cat ggc tgg aac tat gtt aaa gtt tgc cat gct att tgc tgt gtt Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val 370 tta aac aga caa gga ggc aaa aga act act gtt tta ttt cat gga cca Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 gcc aga cac ggc aga tct att att gca caa gca caa gca ggt ggc aga 1248 Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val	Met	cag Gln	cca Pro	gac Asp	agt Ser	Tyr	att Ile	gaa Glu	atg Met	atg Met	Ala	caa Gln	cca Pro	ggt Gly	gga Gly	Glu		960
Thr Lys Thr Åla Phe Åsp Leu Ile Leu Ğlu Lys Åla Ğlu Thr Ser Lys 340 350 350 cta acc aac ttt tca ctg cct gac aca aga acc tgc aga att ttt gct Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala 350 365 ctt cat ggc tgg aac tat gta aa gtt tgc cat gct att tgc tgt gtt Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val 370 375 cta aac aga caa gga ggc aaa aga aat act gtt tta ttt cat gga cca Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 390 400 gcc agc aca ggc aaa tct att att gca caa gcc ata gca caa ggc gt 1248 Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val	aac Asn	ctg Leu	ctg Leu	aaa Lys	Asn	acg Thr	cta Leu	gag Glu	att Ile	Cys	aca Thr	cta Leu	act Thr	cta Leu	Ala	aga Arg	1	.008
Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala 355 365 ttt cat ggc tgg aac tat gtt aaa gtt tgc cat gct att tgc tgt gtt Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val 370 380 tta aac aga caa gga ggc aaa aga aat act gtt tta ttt cat gga cca Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 390 400 gcc agc aca ggc aaa tct att att gca caa ggc ata gca caa ggc gtt 1248 Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val				Āla					Leu					Thr			1	.056
Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val 370 tta aac aga caa gga ggc aaa aga aat act gtt tta ttt cat gga cca Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 gcc agc aca ggc aaa tct att att gca caa gcc ata gca caa gca gtt Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val	cta Leu	acc Thr	Asn	ttt Phe	tca Ser	ctg Leu	cct Pro	Asp	aca Thr	aga Arg	acc Thr	tgc Cys	Arg	att Ile	ttt Phe	gct Ala	1	.104
Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 400 gcc agc aca ggc aca tct att att gca caa gcc ata gca caa gga gtt Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val	ttt Phe	His	ggc Gly	tgg Trp	aac Asn	tat Tyr	Val	aaa Lys	gtt Val	tgc Cys	cat His	Ala	att Ile	tgc Cys	tgt Cys	gtt Val	1	.152
Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val	Leu	aac Asn	aga Arg	caa Gln	gga Gly	Gly	aaa Lys	aga Arg	aat Asn	act Thr	Val	tta Leu	ttt Phe	cat His	gga Gly	Pro	1	.200
	gcc Ala	agc Ser	aca Thr	ggc Gly	Lys	tct Ser	att Ile	att Ile	gca Ala	Gln	gcc Ala	ata Ile	gca Ala	caa Gln	Ala	gtt Val	1	.248

3

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cag Gln	aac Asn	tat Tyr 595	gca Ala	cta Leu	act Thr	cca Pro	ctt Leu 600	gca Ala	tcg Ser	gat Asp	ctc Leu	gag Glu 605	gac Asp	ctg Leu	gct Ala	1824	
tta Leu	gag Glu 610	cct Pro	tgg Trp	agc Ser	aca Thr	cca Pro 615	aat Asn	act Thr	cct Pro	gtt Val	gcg Ala 620	ggc Gly	act Thr	gca Ala	gaa Glu	1872	
acc Thr 625	cag Gln	aac Asn	act Thr	ggg Gly	gaa Glu 630	gct Ala	ggt Gly	tcc Ser	aaa Lys	gcc Ala 635	tgc Cys	caa Gln	gat Asp	ggt Gly	caa Gln 640	1920	
ctg Leu	agc Ser	cca Pro	act Thr	tgg Trp 645	tca Ser	gag Glu	atc Ile	gag Glu	gag Glu 650	gat Asp	ttg Leu	aga Arg	gcg Ala	tgc Cys 655	ttc Phe	1968	
ggt Gly	gcg Ala	gaa Glu	ccg Pro 660	ttg Leu	aag Lys	aaa Lys	gac Asp	ttc Phe 665	agc Ser	gag Glu	ccg Pro	ctg Leu	aac Asn 670	ttg Leu	gac Asp	2016	
taa																2019	

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As nLeu Leu Lys As nThr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 325 330 335Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys 340 345 350 Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cvs Arg Ile Phe Ala 355 360 365 Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 390 395 400 Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val405 410 415 Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn 420 425 430 Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe 435 440 445 Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile 450 460Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro 465 470 480 Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys 485 490 495 Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn 500 505 510 Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys 515 520 525 Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln $530 \hspace{1.5cm} 535 \hspace{1.5cm} 540 \hspace{1.5cm}$ Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 550 555 560 Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu 565 570 575 Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 580 585 590 Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 595 600 605 Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu 610 620 Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 625 630 635 640Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe $645 \hspace{1.5cm} 650 \hspace{1.5cm} 655$ Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asn Leu Asp 660 665 670

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	l> CI	os L)	(2016	5)													
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tta Leu	aag Lys	gaa Glu	aaa Lys 20	agt Ser	aac Asn	cag Gln	gaa Glu	gtg Val 25	ttc Phe	tca Ser	ttt Phe	gtt Val	ttt Phe 30	aaa Lys	aat Asn		96
gaa Glu	aat Asn	gtt Val 35	caa Gln	ctg Leu	aat Asn	gga Gly	aaa Lys 40	gat Asp	atc Ile	gga Gly	tgg Trp	aat Asn 45	agt Ser	tac Tyr	aaa Lys		144
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act Thr 65	act Thr	tgg Trp	gac Asp	caa Gln	agc Ser 70	gag Glu	gac Asp	atg Met	gaa Glu	tgg Trp 75	gaa Glu	acc Thr	aca Thr	gtg Val	gat Asp 80		240
gaa Glu	atg Met	acc Thr	aaa Lys	aag Lys 85	caa Gln	gta Val	ttc Phe	att Ile	ttt Phe 90	gat Asp	tct Ser	ttg Leu	gtt Val	aaa Lys 95	aaa Lys		288
tgt Cys	tta Leu	ttt Phe	gaa Glu 100	gtg Val	ctt Leu	aac Asn	aca Thr	aag Lys 105	aat Asn	ata Ile	ttt Phe	cct Pro	ggt Gly 110	gat Asp	gtt Val		336

aat Asn	tgg Trp	ttt Phe 115	gtg Val	caa Gln	cat His	gaa Glu	tgg Trp 120	gga Gly	aaa Lys	gac Asp	caa Gln	ggc Gly 125	tgg Trp	cac His	tgc Cys		384
							gac Asp										432
							tac Tyr										480
tgt Cys	aat Asn	gtg Val	caa Gln	cta Leu 165	aca Thr	cca Pro	gct Ala	gaa Glu	aga Arg 170	att Ile	aaa Lys	cta Leu	aga Arg	gaa Glu 175	ata Ile		528
gca Ala	gaa Glu	gac Asp	aat Asn 180	gag Glu	tgg Trp	gtt Val	act Thr	cta Leu 185	ctt Leu	act Thr	tat Tyr	aag Lys	cat His 190	aag Lys	caa Gln		576
acc Thr	aaa Lys	aaa Lys 195	gac Asp	tat Tyr	acc Thr	aag Lys	tgt Cys 200	gtt Val	ctt Leu	ttt Phe	gga Gly	aac Asn 205	atg Met	att Ile	gct Ala		624
tac Tyr	tat Tyr 210	ttt Phe	tta Leu	act Thr	aaa Lys	aag Lys 215	aaa Lys	ata Ile	agc Ser	act Thr	agt Ser 220	cca Pro	cca Pro	aga Arg	gac Asp		672
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cgg Arg	cca Pro	gaa Glu	acg Thr 260	gtt Val	gaa Glu	acc Thr	aca Thr	gta Val 265	acc Thr	act Thr	gcg Ala	cag Gln	gaa Glu 270	act Thr	aag Lys		816
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							gaa Glu										960
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the act agg cas ggs agg as agg ags at act gft the the cat ggs cca Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 gcc agc aca ggc aaa tot at att att gca cas gcc att gca cas gcs gtt Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Gln Ala Val 405 ggc aat gft ggt tgc tat aat gca gcc aat gta act tt cca tht aat 1296 Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn 425 gac tgt acc aac aag aac ttg att tgg gta gaa gaa gct ggt aac tht Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe 435 gga cag caa gta aac cag the lie Trp Val Glu Glu Ala Gly Asn Phe 450 gga cag caa gta aac cag the lie Tys Ala Ile Cys Ser Gly Gln Thr Ile 450 cga att gat caa aaa gga aaa ggc agc agc att gaa cca act act alt Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile 460 cga att gat caa aaa gga aaa ggc agc aga att gaa cca aca cca Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro 465 gtc atc atg acc aca aat gag aac att aca gtg gtc aga ata ggc tgc Val Ile Met Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys 475 gaa gaa aga cca gaa cac act caa cca act cag gg gac aga atg ctt aac Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn 510 att cat cta aca cat act tgc cct ggt gac ttt ggt ttg gtt gac aaa 1584 lie His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys 515 sat gaa tgg cca ag att tgt cgt tgg ttg aaa gat ggt tac caa and and gal ash gdt acc caa act caa act caa cat act tgc cct ggt tgg ttg act aga att gac tac caa and san Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln 530 tct acc atg gca agc tac tgt gct agc caa tct cat act act acc aga agg act tgg gc aaa ttt cac act act gga act tgg gag caa agt ctt ggt tgg act act act acc act acc tgg aca act gag gag act act tact acc act acc tgg aca act gag gag act act tact acc act acc tact acc act act																	
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Gily Asn Val Gily Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn 420 425 436 435 436 436 436 435 436					Lys					Gln					Āla		1248
Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe 435 gga cag caa gta aac cag ttt aaa gcc att tgc tct ggt caa act att 450 cgc att gat caa aaa gga aaa ggc agc aaa cag att gaa cca aca cca Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro 465 ggc att gat caa aaa gga aaa ggc agc aaa cag att gaa cca aca cca Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro 465 gtc atc atg acc aca aat gag aca att aca gtg gtc aga ata ggc tgc Val Ile Met Tr Thr Asn Glu Asn Ile Thr Val Arg Ile Gly Cys 488 gaa gaa aga cca gaa cac act caa cca act aga gac aga atg ctt aac Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn 500 att cat cta aca cat acc ttg cct ggt gac ttt ggt ttg gtt gac aaa Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys 515 aat gaa tgg ccc atg att tgt gct tgg ttg gta aag aat ggt tac caa Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln 533 tct acc atg gca agc tac tgt gct aaa tgg gg caa agt cct gat tgg Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 cag aaa act tgg gcg gc ca ag gtg cca aac gtg cca act cct ata aat tta cta Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Ser Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ala Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Ser Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ala Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Ser Glu Asn Trp Ala Glu Asn Ser Asp Leu Glu Asp Leu Ala Glu Asn Try Ala Leu Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu Glu Asn Try Ala Glu Asg Cac acc at acc acc aga gac ct gcg acc gc gac ct gcg Cac acc acc acc acc gag acc gcg gcc cac gcg gac ct gcg Gac cca acc gac acc gca gac gcc gcg Gacc gcc gac gcc gcc gcc gcc gcc gcc gcc				Gly					Ala					Pro			1296
Gly Gln Gln Val Asn Gln Phe Lys Åla Ile Cys Ser Gly Gln Thr Ile 450 cgc att gat caa aaa gga aaa ggc agc aaa cag att gaa cca aca cca Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro 480 gtc atc atg acc aca aat gag aac att aca gtg gtc aga ata ggc tgc Val Ile Met Thr Hs Ash Glu Asn Ile Thr Val Val Arg Ile Gly Cys gaa gaa aga cca gaa cac act caa cca atc aga gac aga atg ctt aac Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn 500 att cat cta aca cat acc ttg cct ggt gac ttt ggt ttg gtt gac aaa Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys 530 att gaa tgg ccc atg att tgt gct tgg ttg gta aag aat ggt aca Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln 531 tct acc atg gca agc tac tgt gct aaa tgg gg aaa ggt cct gat gg Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 tca gaa aac tgg gcg gag cca aag gtg cca aac ggt cca act cct ata aat tta cta Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu 565 ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt acc ctc tca ga Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 580 cag aac tat gga cac acc caa at cca ttg gca cac gca gac ttc gg gga act gca gac Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu 590 cag aac tat gga cac acc act cac tt gca tcg gat ctc gag gac ctc ctc ag Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 580 cag aac tat gga cac acc aca at acc ctt gca tcg gat ctc gag gac ctg gcl Gln Asn Tyr Ala Leu Thr Pro Asn Thr Pro Val Ala Glu Thr Ala Glu 610 acc cag acc act gg gag cac aca acc act gac gac gac ctg gac Cad acc act gg gac acc cac acc act acc gac gac Cad acc acc gac acc gac acc cac acc acc acc			Thr					Ile					Ala				1344
Arg 11e Asp Gln Lys Gly Lys Gly Ser Lys Gln 11e Glu Pro Thr Pro 486 gtc atc atg acc aca aat gag aac att aca gtg gtc aga ata ggc tgc Val I1e Met Thr Thr Asn Glu Asn I1e Thr Val Val Arg I1e Gly Cys gaa gaa aga cca gaa cac act caa cca atc aga gac aga atg ctt aac Glu Glu Arg Pro Glu His Thr Gln Pro 11e Arg Asp Arg Met Leu Asn 500 att cat cta aca cat acc ttg cct ggt gac ttt ggt ttg gtt gac aaa I1e His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys 535 aat gaa tgg ccc atg att tgt gct tgg ttg gta aag aat ggt ac caa Asn Glu Trp Pro Met I1e Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln 535 tct acc atg gca agc tac tgt gct aaa tgg ggc aaa ggt cct ggt gse Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 tca gaa aac tgg gcg gag cca aag gtg cca aact cct ata aat tta cta Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro I1e Asn Leu Leu 565 ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt acg ct ctc ag Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 580 cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctt gca Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asn Leu Ala 600 cag acc tat gga cac acc aat cct ggt gca gcc gc gac act gca gac Cln Asn Tyr Ala Clu Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 610 acc cag aac act tgg gag gag cca aat cct gtt gcg gcg act gca gac Cag aac act gg gag acc aca act cct gtt gcg gcg act gca gac Cag aac act gg gag acc aca acc aat act cct gtt gcg gca ct gca gaa Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu 610 acc cag aac act ggg gag cca act gag act gcg act gcg act Cad acc cad acc act ggg act gcd tcc acc Cad acc act ggg gac cca act gag gat gcd gcd ctc Cad acc ca acc tcd gag act gcd tcc acc Cad acc act ggg gac cca act gag gac ct gcd acc Cad acc act gag gac gcd gcd tcc acc Cad acc act gag gac gcd gcd tcc acc Cad acc acc acc acc acc acc acc acc acc a		Gln					Phe					Ser					1392
Val Ile Met Thr Thr Ash Glu Ash Ile Thr Val Arg Ile Gly Cys gaa gaa aga cca gaa cac act caa cca atc aga gac aga atg ctt aac Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Ash 500 att cat cta aca cat acc ttg cct ggt gac ttt ggt ttg gtt gac aaa Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys 515 aat gaa tgg ccc atg att tgt gct tgg ttg gta aag aat ggt tac caa Ash Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Ash Gly Tyr Gln 530 tct acc atg gca agc tac tgt gct aaa tgg ggc aaa gtt cct gat tgg Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 tca gaa aac tgg gcg gag cca asg gtg cca aat cct ata aat tta cta Ser Glu Ash Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Ash Leu Leu 565 ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt ac ct ctc ag Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 580 cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctg gct Gln Ash Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 600 cag acc tat gga cac acc aat act cct gtt gcg gga act gca ga 1872 tta gag cct tgg acc aca aca at act cct gtt gcg gga act gca ga 1872 tta gag cct tgg acc aca aca aat act cct gtt gcg gga act gca ga 1872 tta gag cct tgg acc aca cca aat act cct gtt gcg gga act gca ga 1872 tta gag cct tgg acc aca cca aat act cct gtt gcg gca act gca ga 1872 tta gag cct tgg acc aca cca aat act cct gtt gcg gca act gca ga 1872 tta gag cct tgg acc aca cca aat act cct gtt gcg gca act gca ga 1872 tta gag cct tga acc aca aca at act cct gtt gcg gca cat gca ga 1872 acc cag aac act ggg gaa gct ggt tcc aaa 1920 ccg acc ca acc act ggg ga gct gdt tcc aaa 1920 ccg acc ca acc tt gg gc acc acc aca at act cct gtt gcg gca ct gcd gca 1920 ccg acc cca acc tt gcg tca gad gct gdt tcc aaa 1920 ccg acc cca acc tt gcg tca gaa gct gdt tcc aaa 1920 ccg acc cca acc tt gcg tca gaa gct gdt tcc aaa 1920 ccg acc cca acc tt gcg tca ga gct gdt tcc aaa 1920 ccg acc cca acct tcg fca ga acc gaa gat gct tcc 1968	Arg					Gly					Gln					Pro	1440
Sin Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn 500 stores 50	gtc Val	atc Ile	atg Met	acc Thr	Thr	aat Asn	gag Glu	aac Asn	att Ile	Thr	gtg Val	gtc Val	aga Arg	ata Ile	Gly	tgc Cys	1488
Ile His Leu Thr His Thr Leu Pro Öly Asp Phe Öly Leu Üul Asp Lys 525 aat gaa tog ccc atg att tgt gct tgg tta gaa aag aat ggt tac caa Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln 530 tct acc atg gca agc tac tgt gct aaa tgg ggc aaa gtt cct gat tgg Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 tca gaa aac tgg gcg gag cca aag gtg cca act cct ata aat tta cta 565 tca gaa aac tgg gcg gag cca aag gtg cca act cct ata aat tta cta 575 ggt tcg gca cgc tca cca ttc acg aca acg aaa agt acg cct cct agc Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Ile Asn Leu Leu Ser 585 cag aac tat gca cta act cca ctt gca tcg atc ggt cct acg gac ctg gct Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 595 tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gg gac tg Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 610 cc aga ac tat gca gca cca cca ata act cct gtt gcg ggc act gca gaa 1872 tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gca gaa 1872 acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt caa fbr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 625 cct gac cca act tgg tca gag atc gag ags gg gat ttg aga gct gct tct 1968 ctg agc cca act tgg tca gag atc gag ags gg att tg aga gct gct tct 1968 Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe	gaa Glu	gaa Glu	aga Arg	Pro	gaa Glu	cac His	act Thr	caa Gln	Pro	atc Ile	aga Arg	gac Asp	aga Arg	Met	ctt Leu	aac Asn	1536
Asn Silu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Sily Tyr Gin 530 tct acc atg gca agc tac tgt gct aaa tgg ggc aaa gtt cct gat tgg Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 tca gaa aac tgg gcg gag cca aag gtg cca act cct ata aat tta cta 565 tca gaa aac tgg gcg gag cca aag gtg cca act cct ata aat tta cta 575 ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt acg cct cct agc Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Ile Asn Leu Leu Ser 585 cag aac tat gca cta cca ttc acg aca ccg aaa agt acg cct cct agc Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 585 cag aac tat gca cta act cca cta tc gca tcg gat ctc gga gac ctg gct Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 600 tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gca gaa 1872 Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu 615 acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt caa 725 fr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 640 ctg agc cca act tgg tca gag atc gag aga gat ttg aga gcg tgt tcc 1968 ctg agc cca act tgg tca gag atc gag aga gat ttg aga gcg tgt tcc 1968 Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe			Leu					Pro					Leu				1584
Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 tca gaa aac tgg gcg gag cca aag gtg cca act cct ata aat tta cta Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu 565 ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt acg cct ctc agc Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 580 cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctg gct Gln Asp Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 555 tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gc act gca gac leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu 615 acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt caa from 610 Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 625 ctg agc cca act tgg tca gag atc gag gag gat ttg aga gct ggt tcc 1968 ctg agc cca act tgg tca gag atc gag aga gat ttg aga gcg tgc ttc 1968 ctg agc cca act tgg tca gag atc gag aga gat ttg aga gcg tgc ttc 1968	aat Asn	Glu	tgg Trp	ccc Pro	atg Met	att Ile	Cys	gct Ala	tgg Trp	ttg Leu	gta Val	Lys	aat Asn	ggt Gly	tac Tyr	caa Gln	1632
Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu 565 ggt tcg gca cgc tca cca ttc acg aca ccg aaa agt acg cct ctc agc Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 580 cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctg gct Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 595 tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gca gaa 1872 teu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu 610 acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt gc act gca gaa 1920 Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 625 ctg agc cca act tgg tca gag atc gag gag gat ttg aga gct gct tcc 1968 ctg agc cca act tgg tca gag atc gag aga gtt gat tga gag gct gct tcc 1968 ctg agc cca act tgg tca gag atc gag aga gtt gat tga gag gct gct tcc 1968	Ser	acc Thr	atg Met	gca Ala	agc Ser	Tyr	tgt Cys	gct Ala	aaa Lys	tgg Trp	Gly	aaa Lys	gtt Val	cct Pro	gat Asp	Trp	1680
Cag aac tat gca cta act cca ctt gca tcg gat ctc gag gac ctg gct cla Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 605 tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gca gaa leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu 615 acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt caa far Glu Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 640 ctg agc cca act tgg tca gag atc gag gag gat ttg aga gcg tgt tcc leu Ser Pro Trp Trp Ser Glu Tle Glu Glu Asp Leu Arg Ala Cys Phe	tca Ser	gaa Glu	aac Asn	tgg Trp	Ala	gag Glu	cca Pro	aag Lys	gtg Val	Pro	act Thr	cct Pro	ata Ile	aat Asn	Leu	cta Leu	1728
Cln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Clu Asp Leu Ala 595 tta gag cct tgg agc aca cca aat act cct gtt gcg ggc act gca gaa Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu 615 acc cag aac act ggg gaa gct ggt tcc aaa gcc tgc caa gat ggt caa Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 625 ctg agc cca act tgg tca gag atc gag agg gat ttg aga gcg tgct tcc 1968 ctg agc cca act tgg tca gag atc gag gag ttg aga gcg tgct ttc 1968 Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe	ggt Gly	tcg Ser	gca Ala	Arg	tca Ser	cca Pro	ttc Phe	acg Thr	Thr	ccg Pro	aaa Lys	agt Ser	acg Thr	Pro	ctc Leu	agc Ser	1776
Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu 610 620 620 620 620 620 620 620 620 620 62	cag Gln	aac Asn	Tyr	gca Ala	cta Leu	act Thr	cca Pro	Leu	gca Ala	tcg Ser	gat Asp	ctc Leu	Glu	gac Asp	ctg Leu	gct Ala	1824
Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 625 630 640 ctg agc cca act tgg tca gag atc gag gag gat ttg aga gcg tgc ttc Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe	tta Leu	Glu	cct Pro	tgg Trp	agc Ser	aca Thr	Pro	aat Asn	act Thr	cct Pro	gtt Val	Ala	ggc Gly	act Thr	gca Ala	gaa Glu	1872
Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe	Thr	cag Gln	aac Asn	act Thr	ggg Gly	Glu	gct Ala	ggt Gly	tcc Ser	aaa Lys	Ala	tgc Cys	caa Gln	gat Asp	ggt Gly	Gln	1920
	ctg Leu	agc Ser	cca Pro	act Thr	Trp	tca Ser	gag Glu	atc Ile	gag Glu	Glu	gat Asp	ttg Leu	aga Arg	gcg Ala	Cys	ttc Phe	1968

ggt gcg gaa ccg ttg aag aaa gac ttc agc gag ccg ctg aac ttg gac Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asp 660 660 665 670

2016

taa

2019

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<213> part of Parvovirus NS1 variant

<400> 5

Thr Ser Pro Glu

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<400> 6

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Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys 35 40 45

Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu 50 55 60

Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp 65 70 75 80

Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys 85 90 95

Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val

Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys 115

His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp 130 135 140

Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala 145 150 155 160

Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile 165 170 175

Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln $180 \hspace{1.5cm} 185 \hspace{1.5cm} 185 \hspace{1.5cm} 190 \hspace{1.5cm}$

Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp 210 215 220 Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu 225 236 239 240 Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met 245 250 255 Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ala Ile Lys Thr Thr Leu 275 280 285 Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met 290 295 300 Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Glu 305 310 315 320 Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 325 330 335 Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys 340 345 350Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala 355 360 365 Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val 370 375 380 Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 390 395 400 Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val 405 410 415 Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn 420 425 430Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile 450 455 460 Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro 465 470 475 480 Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys
485 490 495 Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn $500 \hspace{1.5cm} 505 \hspace{1.5cm} 510 \hspace{1.5cm}$ Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys 515 520 525 Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln
530 540 11

Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 550 555 Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro
580 585 590 Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala
595 600 605 Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asn Leu Asp <210> 7 <211> 60 <212> DNA <213> part of Parvovirus NS1 variant <220> <221> CDS <222> (1)..(60) <400> 7 aca aga gcc tgc aga att ttt gct ttt cat ggc tgg aac tat gtt aaa Thr Arg Ala Cys Arg Ile Phe Ala Phe His Gly Trp Asn Tyr Val Lys 60 gtt tgc cat gct Val Cys His Ala <210> 8 <211> 2019 <212> DNA <213> Parvovirus NS1 variant <220> <221> CDS <222> (1)..(2016) <400> 8 48 atg gct gga aat gct tac tct gat gaa gtt ttg gga gca acc aac tgg Met Ala Gly Asn Ala Tyr Ser Asp Glu Val Leu Gly Ala Thr Asn Trp tta aag gaa aaa agt aac cag gaa gtg ttc tca ttt gtt ttt aaa aat 96 Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn

25

gaa Glu	aat Asn	gtt Val 35	caa Gln	ctg Leu	aat Asn	gga Gly	aaa Lys 40	gat Asp	atc Ile	gga Gly	tgg Trp	aat Asn 45	agt Ser	tac Tyr	aaa Lys	1	44
aaa Lys	gag Glu 50	ctg Leu	cag Gln	gag Glu	gac Asp	gag Glu 55	ctg Leu	aaa Lys	tct Ser	tta Leu	caa Gln 60	cga Arg	gga Gly	gcg Ala	gaa Glu	1	92
act Thr 65	act Thr	tgg Trp	gac Asp	caa Gln	agc Ser 70	gag Glu	gac Asp	atg Met	gaa Glu	tgg Trp 75	gaa Glu	acc Thr	aca Thr	gtg Val	gat Asp 80	2	40
	atg Met															2	88
tgt Cys	tta Leu	ttt Phe	gaa Glu 100	gtg Val	ctt Leu	aac Asn	aca Thr	aag Lys 105	aat Asn	ata Ile	ttt Phe	cct Pro	ggt Gly 110	gat Asp	gtt Val	3	36
	tgg Trp															3	84
	gta Val 130															4	32
	aga Arg															4	80
	aat Asn															5	28
	gaa Glu															5	76
	aaa Lys															6	24
	tat Tyr 210															6	72
	ggc Gly															7	20
aaa Lys	gaa Glu	ggc Gly	gag Glu	cgc Arg 245	cat His	cta Leu	gtg Val	agc Ser	aaa Lys 250	cta Leu	tac Tyr	act Thr	gat Asp	gac Asp 255	atg Met	7	68
cgg Arg	cca Pro	gaa Glu	acg Thr 260	gtt Val	gaa Glu	acc Thr	aca Thr	gta Val 265	acc Thr	act Thr	gcg Ala	cag Gln	gaa Glu 270	act Thr	aag Lys	8	16
	ggc Gly															8	64

aaa Lys	gag Glu 290	ctg Leu	gtg Val	cat His	aaa Lys	aga Arg 295	gta Val	acc Thr	tca Ser	cca Pro	gag Glu 300	gac Asp	tgg Trp	atg Met	atg Met		912	
atg Met 305	cag	cca Pro	gac Asp	agt Ser	tac Tyr 310	att	gaa Glu	atg Met	atg Met	gct Ala 315	caa	cca Pro	ggt Gly	gga Gly	gaa Glu 320		960	
aac	ctg Leu	ctg Leu	aaa Lys	aat Asn 325	acg	cta Leu	gag Glu	att Ile	tgt Cys 330	aca	cta Leu	act Thr	cta Leu	gcc Ala 335	aga		1008	
acc Thr	aaa Lys	aca Thr	gca Ala 340	ttt Phe	gac Asp	tta Leu	att Ile	tta Leu 345	gaa Glu	aaa Lys	gct Ala	gaa Glu	acc Thr 350	agc Ser	aaa Lys		1056	
								aca Thr									1104	
								gtt Val									1152	
tta Leu 385	aac Asn	aga Arg	caa Gln	gga Gly	ggc Gly 390	aaa Lys	aga Arg	aat Asn	act Thr	gtt Val 395	tta Leu	ttt Phe	cat His	gga Gly	cca Pro 400		1200	
gcc Ala	agc Ser	aca Thr	ggc Gly	aaa Lys 405	tct Ser	att Ile	att Ile	gca Ala	caa Gln 410	gcc Ala	ata Ile	gca Ala	caa Gln	gca Ala 415	gtt Val	-	1248	
ggc Gly	aat Asn	gtt Val	ggt Gly 420	tgc Cys	tat Tyr	aat Asn	gca Ala	gcc Ala 425	aat Asn	gta Val	aac Asn	ttt Phe	cca Pro 430	ttt Phe	aat Asn		1296	
								tgg Trp									1344	
gga Gly	cag Gln	caa Gln 450	gta Val	aac Asn	cag Gln	ttt Phe 455	aaa Lys	gcc Ala	att Ile	tgc Cys	tct Ser 460	ggt Gly	caa Gln	act Thr	att Ile		1392	
cgc Arg 465	att Ile	gat Asp	caa Gln	aaa Lys	gga Gly 470	aaa Lys	ggc Gly	agc Ser	aaa Lys	cag Gln 475	att Ile	gaa Glu	cca Pro	aca Thr	cca Pro 480		1440	
gtc Val	atc Ile	atg Met	acc Thr	aca Thr 485	aat Asn	gag Glu	aac Asn	att Ile	aca Thr 490	gtg Val	gtc Val	aga Arg	ata Ile	ggc Gly 495	tgc Cys		1488	
								cca Pro 505									1536	
								ggt Gly									1584	
aat Asn	gaa Glu 530	tgg Trp	ccc Pro	atg Met	att Ile	tgt Cys 535	gct Ala	tgg Trp	ttg Leu	gta Val	aag Lys 540	aat Asn	ggt Gly	tac Tyr	caa Gln		1632	
tct Ser 545	acc Thr	atg Met	gca Ala	agc Ser	tac Tyr 550	tgt Cys	gct Ala	aaa Lys	tgg Trp	ggc Gly 555	aaa Lys	gtt Val	cct Pro	gat Asp	tgg Trp 560		1680	

					gtg Val				1728	
					aca Thr 585				1776	
_					gca Ala				1824	
					act Thr				1872	
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					gag Glu				1968	
					ttc Phe 665				2016	
	taa								2019	

<210> 9 <211> 20

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Thr Arg Ala Cys Arg Ile Phe Ala Phe His Gly Trp Asn Tyr Val Lys 10

Val Cys His Ala 20

<210> 10

<211> 672 <212> PRT

<213> Parvovirus NS1 variant

<400> 10

Met Ala Gly Asn Ala Tyr Ser Asp Glu Val Leu Gly Ala Thr Asn Trp $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn 20 25 30Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu 50 55 60

<212> PRT

<213> part of Parvovirus NS1 variant

Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp 65 70 75 80 Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys 85 90 95 Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Vel Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys 115 120 125 His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp 130 135 140 Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala 145 150 155 160 Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile 165 170 175 Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln
180 185 190 Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala 195 200 205 Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp 210 215 220 Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu 225 230 235 240 Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met 245 250 255 Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys 260 265 270Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu 275 280 285 Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met 290 300Met Gln Pro Asp Ser Tyr Ile Glu Met Met Ala Gln Pro Gly Glu 305 310 315 320 Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 325 330 335Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys 340 345 350Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Ala Cys Arg Ile Phe Ala 355 360 365 Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val 370 380 Leu Asn Arg Gln Gly Gly Lys Arg Asn Thr Val Leu Phe His Gly Pro 385 390 395 400

Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val 405 410 415

Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn 420 425

Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe

Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile

Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro

Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys 485 490 495

Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn

Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys 515 520 525

Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln 530 540

Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 550 560

Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu 565 570 575

Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 580 590

Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala 595 600 605

Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu 610 615 620

Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 625 630 635

Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe 645 650 655

Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asn Leu Asp

<210> 11

<211> 60 <212> DNA

<213> part of Parvovirus NS1 variant

<220>

<221> CDS <222> (1) .. (60)

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1

ttt cat gga cca Phe His Gly Pro 20 60

	<211	> 12 > 20	19														
	<212 <213	> DN > Pa	rvov	irus	NS1	var	iant										
		> > CE > (1		2016)												
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	tta Leu	aag Lys	gaa Glu	aaa Lys 20	agt Ser	aac Asn	cag Gln	gaa Glu	gtg Val 25	ttc Phe	tca Ser	ttt Phe	gtt Val	ttt Phe 30	aaa Lys	aat Asn	96
	Glu	aat Asn	Val 35	Gln	Leu	Asn	GIY	40	Asp	116	GIY	11p	45	561	-,-	-,-	144
	Lys	gag Glu 50	Leu	Gln	Glu	Asp	55 55	Leu	гуѕ	Ser	Бец	60	my	01,			192
	Thr 65	act Thr	Trp	Asp	Gln	Ser 70	GIu	Asp	Mec	GIU	75	Giu	1111		V 41	80	240
	gaa Glu	atg Met	acc Thr	aaa Lys	aag Lys 85	caa Gln	gta Val	ttc Phe	att Ile	ttt Phe 90	gat Asp	tct Ser	ttg Leu	gtt Val	aaa Lys 95	aaa Lys	288
	tgt Cys	tta Leu	ttt Phe	gaa Glu 100	gtg Val	ctt Leu	aac Asn	aca Thr	aag Lys 105	aat Asn	ata Ile	ttt Phe	cct Pro	ggt Gly 110	gat Asp	gtt Val	336
	aat Asn	tgg Trp	ttt Phe 115	gtg Val	caa Gln	cat His	gaa Glu	tgg Trp 120	gga Gly	aaa Lys	gac Asp	caa Gln	ggc Gly 125	tgg Trp	cac His	tgc Cys	384
	His	gta Val 130	Leu	Ile	Gly	Gly	Lys 135	Asp	Pne	Ser	GIII	140	GIII	Cly	2,0		432
•	Trp 145		Arg	Gln	Leu	150	vai	TYL	11p	Ser	155	11.0	202		••••	160	480
•	Cys	Asn	Val	Gln	Leu 165	Thr	Pro	Ala	GIU	170	116	Dys	Dea	ni g	175		528
	gca Ala	gaa Glu	gac Asp	aat Asn 180	gag Glu	tgg Trp	gtt Val	act	cta Leu 185	Deu	act Thr	tat Tyr	aag Lys	cat His 190	-2-	caa Gln	576

acc Thr	Lys	Lys 195	ASP	Tyr	TILL	БУБ	200	•41			2	205				624
tac Tyr	tat Tyr 210	ttt Phe	tta Leu	act Thr	aaa Lys	aag Lys 215	aaa Lys	ata Ile	agc Ser	act Thr	agt Ser 220	cca Pro	cca Pro	aga Arg	gac Asp	672
gga Gly 225	Gly	Tyr	Phe	Leu	230	ser	ASP	Ser	GIY	235	2,5				240	720
aaa Lys	g a a Glu	ggc Gly	gag Glu	cgc Arg 245	cat His	cta Leu	gtg Val	agc Ser	aaa Lys 250	cta Leu	tac Tyr	act Thr	gat Asp	gac Asp 255	atg Met	768
cgg Arg	cca Pro	gaa Glu	acg Thr 260	gtt Val	gaa Glu	acc Thr	aca Thr	gta Val 265	acc Thr	act Thr	gcg Ala	cag Gln	gaa Glu 270	act Thr	aag Lys	816
cgc Arg	ggc Gly	aga Arg 275	att Ile	caa Gln	act Thr	aaa Lys	aaa Lys 280	gaa Glu	gtt Val	tct Ser	att Ile	aaa Lys 285	act Thr	aca Thr	ctt Leu	864
aaa Lys	gag Glu 290	ctg Leu	gtg Val	cat His	aaa Lys	aga Arg 295	gta Val	acc Thr	tca Ser	cca Pro	gag Glu 300	gac Asp	tgg Trp	atg Met	atg Met	912
atg Met 305	cag Gln	cca Pro	gac Asp	agt Ser	tac Tyr 310	att Ile	gaa Glu	atg Met	atg Met	gct Ala 315	caa Gln	cca Pro	ggt Gly	gga Gly	gaa Glu 320	960
aac Asn	ctg Leu	ctg Leu	aaa Lys	aat Asn 325	acg Thr	cta Leu	gag Glu	att Ile	tgt Cys 330	aca Thr	cta Leu	act Thr	cta Leu	gcc Ala 335	aga Arg	1008
acc Thr	aaa Lys	aca Thr	gca Ala 340	Phe	gac Asp	tta Leu	att Ile	tta Leu 345	gaa Glu	aaa Lys	gct Ala	gaa Glu	acc Thr 350	agc Ser	aaa Lys	1056
cta Leu	acc Thr	aac Asn 355	ttt Phe	tca Ser	ctg Leu	cct Pro	gac Asp 360	aca Thr	aga Arg	acc Thr	tgc Cys	aga Arg 365	att Ile	ttt Phe	gct Ala	1104
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tta Leu 385	aac Asn		caa Gln	gga Gly	ggc Gly 390	гys	aga Arg	aat Asn	gct Ala	gtt Val 395		ttt Phe	cat His	gga Gly	Pro 400	1200
gcc Ala	ago	aca Thr	ggc	aaa Lys	Ser	att Ile	att Ile	gca Ala	caa Gln 410		ata Ile	gca Ala	caa Gln	gca Ala 415	gtt Val	1248
ggc Gly	aat Asr	gtt Val	ggt Gl ₃	Cys	tat Tyr	aat Asn	gca Ala	gco Ala 425		gta Val	aac Asr	ttt Phe	cca Pro 430	ttt Phe	aat Asn	1296
gac Asp	tgt Cys	acc Thr 435	: Asr	aag Lys	aac Asr	tto Lev	att 1116 440	117	gta Val	gaa Glu	a gaa 1 Glv	a gct 1 Ala 445	ggt Gly	aac Asr	ttt Phe	1344

gga Gly	cag G1n 450	caa G1n	gta Val	aac Asn	cag Gln	ttt Phe 455	aaa Lys	gcc Ala	att Ile	tgc Cys	tct Ser 460	ggt Gly	caa Gln	act Thr	att Ile	1392
cgc Arg 465_	att Ile	gat Asp	caa Gln	Lys	gga Gly 470	Lys	ggc Gly	Ser	Lys	cag Gln 475	Ile	gaa Glu	cca Pro	aca Thr	cca Pro	1440
	atc Ile	atg Met	acc Thr	aca Thr 485	aat Asn	gag Glu	aac Asn	att Ile	aca Thr 490	gtg Val	gtc Val	aga Arg	ata Ile	ggc G1y 495	tgc Cys	1488
gaa Glu	gaa Glu	aga Arg	cca Pro 500	gaa G1u	cac His	act Thr	caa G1n	cca Pro 505	atc Ile	aga Arg	gac Asp	aga Arg	atg Met 510	ctt Leu	aac Asn	1536
att Ile	cat His	cta Leu 515	aca Thr	cat His	acc Thr	ttg Leu	cct Pro 520	ggt Gly	gac Asp	ttt Phe	ggt Gly	ttg Leu 525	gtt Val	gac Asp	aaa Lys	1584
aat Asn	gaa G1u 530	tgg Trp	ccc Pro	atg Met	att Ile	tgt Cys 535	gct Ala	tgg Trp	ttg Leu	gta Val	aag Lys 540	aat Asn	ggt Gly	tac Tyr	caa G1n	1632
tct Ser 545	acc Thr	atg Met	gca Ala	agc Ser	tac Tyr 550	tgt Cys	gct Ala	aaa Lys	tgg Trp	ggc Gly 555	aaa Lys	gtt Val	cct Pro	gat Asp	tgg Trp 560	1680
tca Ser	gaa Glu	aac Asn	tgg Trp	gcg A1a 565	gag Glu	cca Pro	aag Lys	gtg Val	cca Pro 570	act Thr	cct Pro	ata Ile	aat Asn	tta Leu 575	cta Leu	1728
ggt Gly	tcg Ser	gca Ala	cgc Arg 580	tca Ser	cca Pro	ttc Phe	acg Thr	aca Thr 585	ccg Pro	aaa Lys	agt Ser	acg Thr	cct Pro 590	ctc Leu	agc Ser	1776
cag Gln	aac Asn	tat Tyr 595	gca Ala	cta Leu	act Thr	cca Pro	ctt Leu 600	gca Ala	tcg Ser	gat Asp	ctc Leu	gag Glu 605	gac Asp	ctg Leu	gct Ala	1824
tta Leu	gag Glu 610	cct Pro	tgg Trp	agc Ser	aca Thr	cca Pro 615	aat Asn	act Thr	cct Pro	gtt Val	gcg Ala 620	ggc Gly	act Thr	gca Ala	gaa Glu	1872
acc Thr 625	cag Gln	aac Asn	act Thr	ggg Gly	gaa Glu 630	gct Ala	ggt Gly	tcc Ser	aaa Lys	gcc Ala 635	tgc Cys	caa Gln	gat As p	ggt Gly	caa Gln 640	1920
ctg Leu	agc Ser	cca Pro	act Thr	tgg Trp 645	tca Ser	gag Glu	atc Ile	gag G1u	gag Glu 650	gat Asp	ttg Leu	aga Arg	gcg Ala	tgc Cys 655	ttc Phe	1968
ggt Gly	gcg Ala	gaa G1u	ccg Pro 660	ttg Leu	aag Lys	aaa Lys	gac Asp	ttc Phe 665	agc Ser	gag Glu	ccg Pro	ctg Leu	aac Asn 670	ttg Leu	gac Asp	2016
taa																2019

<210> 13 <211> 20 <212> PRT <213> part of Parvovirus NS1 variant

<400> 13

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Phe His Gly Pro 20

<210> 14 <211> 672

<211> 672 <212> PRT

<213> Parvovirus NS1 variant

<400> 14

Met Ala Gly Asn Ala Tyr Ser Asp Glu Val Leu Gly Ala Thr Asn Trp Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn 20 25 30 Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys 35 40 45Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu 50 60 Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp 65 70 75 80 Glu Met Thr Lys Lys Gln Val Phe Ile Phe Asp Ser Leu Val Lys Lys 85 90 95 Cys Leu Phe Glu Val Leu Asn Thr Lys Asn Ile Phe Pro Gly Asp Val $100 \ \ \, 105 \ \ \, 105$ Asn Trp Phe Val Gln His Glu Trp Gly Lys Asp Gln Gly Trp His Cys 115 120 125 His Val Leu Ile Gly Gly Lys Asp Phe Ser Gln Ala Gln Gly Lys Trp 130 135 140 Trp Arg Arg Gln Leu Asn Val Tyr Trp Ser Arg Trp Leu Val Thr Ala 145 150 150 155 Cys Asn Val Gln Leu Thr Pro Ala Glu Arg Ile Lys Leu Arg Glu Ile 165 170 175Ala Glu Asp Asn Glu Trp Val Thr Leu Leu Thr Tyr Lys His Lys Gln 180 185 Thr Lys Lys Asp Tyr Thr Lys Cys Val Leu Phe Gly Asn Met Ile Ala 195 200 205 Tyr Tyr Phe Leu Thr Lys Lys Lys Ile Ser Thr Ser Pro Pro Arg Asp 210 215 Gly Gly Tyr Phe Leu Ser Ser Asp Ser Gly Trp Lys Thr Asn Phe Leu 225 230 240 Lys Glu Gly Glu Arg His Leu Val Ser Lys Leu Tyr Thr Asp Asp Met 245 250 255 Arg Pro Glu Thr Val Glu Thr Thr Val Thr Thr Ala Gln Glu Thr Lys $260 \hspace{1cm} 265 \hspace{1cm}$ Arg Gly Arg Ile Gln Thr Lys Lys Glu Val Ser Ile Lys Thr Thr Leu $_{275}^{\rm 230}$ Lys Glu Leu Val His Lys Arg Val Thr Ser Pro Glu Asp Trp Met Met 290 295 300 Sim Pro Asp Ser Tyr Fle Sku Met Met Met Ala Sim Pro Sly Sky Sku 310 315 Asn Leu Leu Lys Asn Thr Leu Glu Ile Cys Thr Leu Thr Leu Ala Arg 325 330 335 Thr Lys Thr Ala Phe Asp Leu Ile Leu Glu Lys Ala Glu Thr Ser Lys 340 345 Leu Thr Asn Phe Ser Leu Pro Asp Thr Arg Thr Cys Arg Ile Phe Ala 355Phe His Gly Trp Asn Tyr Val Lys Val Cys His Ala Ile Cys Cys Val 370 375 380 Leu Asn Arg Gln Gly Gly Lys Arg Asn Ala Val Leu Phe His Gly Pro 385 390 395 400 Ala Ser Thr Gly Lys Ser Ile Ile Ala Gln Ala Ile Ala Gln Ala Val 405 410 415 Gly Asn Val Gly Cys Tyr Asn Ala Ala Asn Val Asn Phe Pro Phe Asn 420 425 430 Asp Cys Thr Asn Lys Asn Leu Ile Trp Val Glu Glu Ala Gly Asn Phe
435 Gly Gln Gln Val Asn Gln Phe Lys Ala Ile Cys Ser Gly Gln Thr Ile 450 455 Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile Glu Pro Thr Pro 465 470 475 Val Ile Met Thr Thr Asn Glu Asn Ile Thr Val Val Arg Ile Gly Cys Glu Glu Arg Pro Glu His Thr Gln Pro Ile Arg Asp Arg Met Leu Asn Ile His Leu Thr His Thr Leu Pro Gly Asp Phe Gly Leu Val Asp Lys 515 520 Asn Glu Trp Pro Met Ile Cys Ala Trp Leu Val Lys Asn Gly Tyr Gln 530 540 Ser Thr Met Ala Ser Tyr Cys Ala Lys Trp Gly Lys Val Pro Asp Trp 545 555 555 Ser Glu Asn Trp Ala Glu Pro Lys Val Pro Thr Pro Ile Asn Leu Leu 575 Gly Ser Ala Arg Ser Pro Phe Thr Thr Pro Lys Ser Thr Pro Leu Ser 580 Gln Asn Tyr Ala Leu Thr Pro Leu Ala Ser Asp Leu Glu Asp Leu Ala $595 600 $

Leu Glu Pro Trp Ser Thr Pro Asn Thr Pro Val Ala Gly Thr Ala Glu

22

Thr Gln Asn Thr Gly Glu Ala Gly Ser Lys Ala Cys Gln Asp Gly Gln 630

Leu Ser Pro Thr Trp Ser Glu Ile Glu Glu Asp Leu Arg Ala Cys Phe

Giy kia Giu Pro beu bys bys Asp Phe Ser Giu Pro beu Ash beu Asp 665 670

<210> 15 <211> 60 <212> DNA <213> part of Parvovirus NS1 variant <220> <221> CDS <222> (1)..(60) <400> 15 ggt caa gct att cgc att gat caa aaa gga aaa ggc agc aaa cag att Gly Gln Ala Ile Arg Ile Asp Gln Lys Gly Lys Gly Ser Lys Gln Ile 10 60 gaa cca aca cca Glu Pro Thr Pro 20 <210> 16 <211> 2019 <212> DNA <213> Parvovirus NS1 variant <220> <221> CDS <222> (1)..(2016) <400> 16 atg gct gga aat gct tac tct gat gaa gtt ttg gga gca acc aac tgg Met Ala Gly Asn Ala Tyr Ser Asp Glu Val Leu Gly Ala Thr Asn Trp 48 tta aag gaa aaa agt aac cag gaa gtg ttc tca ttt gtt ttt aaa aat Leu Lys Glu Lys Ser Asn Gln Glu Val Phe Ser Phe Val Phe Lys Asn gaa aat gtt caa ctg aat gga aaa gat atc gga tgg aat agt tac aaa Glu Asn Val Gln Leu Asn Gly Lys Asp Ile Gly Trp Asn Ser Tyr Lys 144 aaa gag ctg cag gag gac gag ctg aaa tct tta caa cga gga gcg gaa Lys Glu Leu Gln Glu Asp Glu Leu Lys Ser Leu Gln Arg Gly Ala Glu 192 240 act act tgg gac caa agc gag gac atg gaa tgg gaa acc aca gtg gat

Thr Thr Trp Asp Gln Ser Glu Asp Met Glu Trp Glu Thr Thr Val Asp

70

gaa Glu	atg Met	acc Thr	aaa Lys	aag Lys 85	caa Gln	gta Val	ttc Phe	att Ile	ttt Phe 90	gat Asp	tct Ser	ttg Leu	gtt Val	aaa Lys 95	aaa Lys		288
tgt Cys	tta Leu	ttt Phe	gaa Glu 100	gtg Val	ctt Leu	aac Asn	aca Thr	aag Lys 105	aat Asn	ata Ile	ttt Phe	cct Pro	ggt Gly 110	gat Asp	gtt Val		336
aat Asn	tgg Trp	ttt Phe 115	gtg Val	caa Gln	cat His	gaa Glu	tgg Trp 120	gga Gly	aaa Lys	gac Asp	caa Gln	ggc Gly 125	tgg Trp	cac His	tgc Cys		384
cat His	gta Val 130	cta Leu	att Ile	gga Gly	gga Gly	aag Lys 135	gac Asp	ttt Phe	agt Ser	caa Gln	gct Ala 140	caa Gln	ggg Gly	aaa Lys	tgg Trp		432
tgg Trp 145	aga Arg	agg Arg	caa Gln	cta Leu	aat Asn 150	gtt Val	tac Tyr	tgg Trp	agc Ser	aga Arg 155	tgg Trp	ttg Leu	gta Val	aca Thr	gcc Ala 160		480
tgt Cys	aat Asn	gtg Val	caa Gln	cta Leu 165	aca Thr	cca Pro	gct Ala	gaa Glu	aga Arg 170	att Ile	aaa Lys	cta Leu	aga Arg	gaa Glu 175	ata Ile		528
gca Ala	gaa Glu	gac Asp	aat Asn 180	gag Glu	tgg Trp	gtt Val	act Thr	cta Leu 185	ctt Leu	act Thr	tat Tyr	aag Lys	cat His 190	aag Lys	caa Gln		576
Thr	aaa Lys	Lys 195	Asp	Tyr	Thr	Lys	200	vai	Leu	Pne	GIY	205	Mec	116	ΛIG		624
tac Tyr	tat Tyr 210	ttt Phe	tta Leu	act Thr	aaa Lys	aag Lys 215	aaa Lys	ata Ile	agc Ser	act Thr	agt Ser 220	cca Pro	cca Pro	aga Arg	gac As p		672
gga Gly 225	ggc Gly	tat Tyr	ttt Phe	ctt Leu	agc Ser 230	agt Ser	gac Asp	tct Ser	ggc Gly	tgg Trp 235	aaa Lys	act Thr	aac Asn	ttt Phe	tta Leu 240		720
aaa Lys	gaa Glu	ggc Gly	gag Glu	cgc Arg 245	cat His	cta Leu	gtg Val	agc Ser	aaa Lys 250	cta Leu	tac Tyr	act Thr	gat Asp	gac Asp 255	atg Met		768
cgg Arg	cca Pro	gaa Glu	acg Thr 260	gtt Val	gaa Glu	acc Thr	aca Thr	gta Val 265	acc Thr	act Thr	gcg Ala	cag Gln	gaa Glu 270	act Thr	aag Lys		816
cgc Arg	ggc Gly	aga Arg 275	att Ile	caa Gln	act Thr	aaa Lys	aaa Lys 280	gaa Glu	gtt Val	tct Ser	att Ile	aaa Lys 285	act Thr	aca Thr	ctt Leu		864
aaa Lys	gag Glu 290	ctg Leu	gtg Val	cat His	aaa Lys	aga Arg 295	gta Val	acc Thr	tca Ser	cca Pro	gag Glu 300	gac Asp	tgg Trp	atg Met	atg Met		912
atg Met 305	cag Gln	cca Pro	gac Asp	agt Ser	tac Tyr 310	att Ile	gaa Glu	atg Met	atg Met	gct Ala 315	caa Gln	cca Pro	ggt Gly	gga Gly	gaa Glu 320		960
aac Asn	ctg Leu	ctg Leu	aaa Lys	aat Asn 325	acg Thr	cta Leu	gag Glu	att Ile	tgt Cys 330	aca Thr	cta Leu	act Thr	cta Leu	gcc Ala 335	aga Arg	:	1008
acc Thr	aa a Lys	aca Thr	gca Ala 340	ttt Phe	gac Asp	tta Leu	att Ile	tta Leu 345	gaa Glu	aaa Lys	gct Ala	gaa Glu	acc Thr 350	agc Ser	aaa Lys	:	1056

ct. Le	a acc	aac Asn 355	ttt Phe	tca Ser	ctg Leu	cct Pro	gac Asp 360	aca Thr	aga Arg	acc Thr	tgc Cys	aga Arg 365	att Ile	ttt Phe	gct Ala	11	.04
tt Ph	t cat e Hi: 37	ggc Gly	tgg Trp	aac Asn	tat Tyr	gtt Val 375	aaa Lys	gtt Val	tgc Cys	cat His	gct Ala 380	att Ile	tgc Cys	tgt Cys	gtt Val	11	.52
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gc Al	c age	aca Thr	ggc Gly	aaa Lys 405	tct Ser	att Ile	att Ile	gca Ala	caa Gln 410	gcc Ala	ata Ile	gca Ala	caa Gln	gca Ala 415	gtt Val	12	248
gg Gl	c aa y Asi	gtt Val	ggt Gly 420	tgc Cys	tat Tyr	aat Asn	gca Ala	gcc Ala 425	aat Asn	gta Val	aac Asn	ttt Phe	cca Pro 430	ttt Phe	aat Asn	12	296
ga As	c tg p Cy:	acc Thr 435	aac Asn	aag Lys	aac Asn	ttg Leu	att Ile 440	tgg Trp	gta Val	gaa Glu	gaa Glu	gct Ala 445	ggt Gly	aac Asn	ttt Phe	13	344
gg Gl	a ca y Gli 45	g caa n Gln	gta Val	aac Asn	cag Gln	ttt Phe 455	aaa Lys	gcc Ala	att Ile	tgc Cys	tct Ser 460	ggt Gly	caa Gln	gct Ala	att Ile	13	392
cg Ar 46	g Il	gat Asp	caa Gln	aaa Lys	gga Gly 470	aaa Lys	ggc Gly	agc Ser	aaa Lys	cag Gln 475	att Ile	gaa Glu	cca Pro	aca Thr	cca Pro 480	14	140
gt Va	c at	atg Met	acc Thr	aca Thr 485	aat Asn	gag Glu	aac Asn	att Ile	aca Thr 490	gtg Val	gtc Val	aga Arg	ata Ile	ggc Gly 495	tgc Cys	14	188
ga G1	a ga u Gl	a aga u Arg	cca Pro 500	gaa Glu	cac His	act Thr	caa Gln	cca Pro 505	atc Ile	aga Arg	gac Asp	aga Arg	atg Met 510	ctt Leu	aac Asn	1	536
at Il	t ca e Hi	t cta s Leu 515	Thr	cat His	acc Thr	ttg Leu	cct Pro 520	ggt Gly	gac Asp	ttt Phe	ggt Gly	ttg Leu 525	gtt Val	gac Asp	aaa Lys	1	584
aa As	t ga n Gl 53	a tgg u Trp	ccc Pro	atg Met	att Ile	tgt Cys 535	gct Ala	tgg Trp	ttg Leu	gta Val	aag Lys 540	aat Asn	ggt Gly	tac Tyr	caa Gln	1	632
to Se 54	r Th	c ato r Met	gca Ala	agc Ser	tac Tyr 550	tgt Cys	gct Ala	aaa Lys	tgg Trp	ggc Gly 555	aaa Lys	gtt Val	cct Pro	gat Asp	tgg Trp 560	1	680
to Se	a ga er Gl	a aac u Asr	tgg Trp	gcg Ala 565	GIU	cca Pro	aag Lys	gtg Val	cca Pro 570	act Thr	cct Pro	ata Ile	aat Asn	tta Leu 575	cta Leu	1	728
gg G1	t to y Se	g gca r Ala	cgc Arg 580	Ser	cca	ttc Phe	acg Thr	aca Thr 585	ccg Pro	aaa Lys	agt Ser	acg Thr	cct Pro 590	ctc Leu	agc Ser	1	776
Ca G1	ig aa In As	c tat n Ty: 59!	: Ala	cta Leu	act Thr	cca Pro	ctt Leu 600	gca Ala	tcg Ser	gat Asp	ctc	gag Glu 605	ASP	ctg Leu	gct Ala	1	824
t t Le	a ga eu Gl 61	g cci u Pro 0	tgg Trp	agc Ser	aca Thr	cca Pro 615	ASI	act Thr	cct Pro	gtt Val	gcg Ala 620		act Thr	gca Ala	gaa Glu	1	872

acc Thr 625	cag Gln	aac Asn	act Thr	Gly ggg	gaa Glu 630	gct Ala	ggt Gly	tcc Ser	aaa Lys	gcc Ala 635	tgc Cys	caa Gln	gat Asp	ggt Gly	caa Gln 640	1920
									~~~	~-+	++~	202	aca	tac	ttc	1968
 ctg	agc	cca Pro	act	tgg	tca	gag	atc	Glu	GIU	ASD	Leu	Arg	Ala	Cys	Phe	
Leu	Ser	PFO	THE	645	261	014			650	-				655		
								++0	200	~aa	cca	cta	aac	tta	gac	2016
ggt	gcg	gaa Glu	ccg	ttg	aag	LAZS	Asp	Phe	Ser	Glu	Pro	Leu	Asn	Leu	Asp	
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	~1	Ala	T1 -	3	T10	Acn	Gln	Lvs	Glv	Lvs	Gly	Ser	Lys	Gln	Ile	
GIA	Gin	Ala	TTE	5	116	пор		-1-	10	-				15		
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Glu	Pro	Thr	Pro 20													
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<21 <21 <21	0> 1 1> 6 2> F 3> F 0> 1	72 RT arvo	viru	s NS	1 va:	rian	t									
Met	Ala	Gly	Asn	Ala	Tyr	Ser	Asp	Glu	Val	Leu	Gly	Ala	Thr	Asn 15	Trp	
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Leu	Lys	Glu	Lys	Ser	Asn	Gln	Glu	. Val	Phe	Ser	Phe	vai	30	: гуз	Asn	
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Glu	Asr	ı Val	Gln	Leu	Asn	Gly	Lys	Asp	Ile	Gly	Trp	Asn	Ser	Tyr	Lys	
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rĀs	5(	) Lec	GIL	GIL	nop	55		_			60	)				
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Thi 65	Th:	r Tr	) Asp	Glr	1 Ser 70	GIU	I ASL	ne	. 610	75	5				80	
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Gli	ı Me	t Th	r Lys	Lys	Glr	val	. Ph€	e Ile	e Ph∈ 90	e Ast	Sei	Let	ı va.	95	Lys	
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Cvs	s Le	u Ph	e Glı	ı Val	Leu	ı Ası	n Thi	Ly	s Asr	ılle	e Pho	Pro	Gl:	y Asi	val	
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ASI	LIE	11	5				120	5				12	Ď			
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Hi:	s Va 13	υ T Fe	u Ile	e GI	A GT	13	5 AS)	لللتاب			14	0		-	_	
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145	Arg				150					133					100
Cys	Asn	Val	Gln	Leu 165	Thr	Pro	Ala	Glu	Arg 170	Ile	Lys	Leu	Arg	Glu 175	Ile
Ala	Glu	Asp	Asn 180	Glu	Trp	Val	Thr	Leu 185	Leu	Thr	Tyr	Lys	His 190	Lys	Gln
Thr	Lys	Lys 195	Asp	Tyr	Thr	Lys	Cys 200	Val	Leu	Phe	Gly	Asn 205	Met	Ile	Ala
Tyr	Tyr 210	Phe	Leu	Thr	Lys	Lys 215	Lys	Ile	Ser	Thr	Ser 220	Pro	Pro	Arg	Asp
Gly 225	Gly	Tyr	Phe	Leu	Ser 230	Ser	Asp	Ser	Gly	Trp 235	Lys	Thr	Asn	Phe	Leu 240
Lys	Glu	Gly	Glu	Arg 245	His	Leu	Val	Ser	Lys 250	Leu	Tyr	Thr	Asp	Asp 255	Met
Arg	Pro	Glu	Thr 260	Val	Glu	Thr	Thr	Val 265	Thr	Thr	Ala	Gln	Glu 270	Thr	Lys
Arg	Gly	Arg 275	Ile	Gln	Thr	Lys	Lys 280	Glu	Val	Ser	Ile	Lys 285	Thr	Thr	Leu
Lys	Glu 290	Leu	Val	His	Lys	Arg 295	Val	Thr	Ser	Pro	Glu 300	Asp	Trp	Met	Met
305	Gln				310					Ala 315					320
Asn	Leu	Leu	Lys	Asn 325	Thr	Leu	Glu	Ile	Cys 330	Thr	Leu	Thr	Leu	Ala 335	Arg
Thr	Lys	Thr	Ala 340	Phe	Asp	Leu	Ile	Leu 345	Glu	Lys	Ala	Glu	Thr 350	Ser	Lys
	Thr	355					360					363			
	His 370					375					360				
385	Asn				390					3,7,5					
	Ser			405					410						
	Asn		420					423					*50		
	Cys	435					440					447			
	Gln 450					455					400				
465					470					4/3					
	Ile			485					490					400	
Glu	Glu	Arg	Pro	Glu	His	Thr	Gln	Pro	Ile	Arg	Asp	Arg	Met	Leu	Asn

| Solution | Solution

Gly Ala Glu Pro Leu Lys Lys Asp Phe Ser Glu Pro Leu Asn Leu Asp

## INTERNATIONAL SEARCH REPORT

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Inc	tional	Application No
PCT	/FP	00/07835

A. CLASSI IPC 7	FICATION OF SUBJECT MATTER C12N15/35 C07K14/015 C07K16/0 A61K35/76 A61K48/00	08 G01N33/569	C12Q1/70								
	o International Patent Classification (IPC) or to both national classific	cation and IPC									
	SEARCHED  commentation searched (classification system followed by classification)	Jan ayant da									
IPC 7	C12N C07K A61K	ion symbols)									
Documenta	tion searched other than minimum documentation to the extent that	such documents are included in the	helds searched								
1	ata base consulted during the international search (name of data ba	ase and, where practical, search term	ns used)								
	ternal, STRAND, MEDLINE, BIOSIS		(4)								
	ENTS CONSIDERED TO BE RELEVANT										
Category *	Citation of document, with indication, where appropriate, of the re	levant passages	Relevant to claim No.								
x	regions of the NS-1 protein of t		1-5,7-11								
1	parvovirus Minute Virus of Mice a involved in cytotoxicity and pro										
1	trans inhibition"	llocer									
	JOURNAL OF VIROLOGY,										
	vol. 66, no. 10, October 1992 (19   pages 5705-5713. XP000867510	992-10),									
	AMERICAN SOCIETY FOR MICROBIOLOGY	y us									
	*mutants pMMBal31 and pULB3201;		•								
	and page 5710 first paragraph*										
		-/									
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X Furti	ner documents are listed in the continuation of box C.	Patent family members an	e listed in annex.								
* Special ca	tegories of cited documents :	*T* later document published after t	the international filing date								
"A" docume consid	ent defining the general state of the art which is not lered to be of particular relevance	or priority date and not in confi cited to understand the princip invention	lict with the application but ile or theory underlying the								
	focument but published on or after the international	"X" document of particular relevance	e; the claimed invention								
	nt which may throw doubts on priority claim(s) or is cited to establish the publication date of another	cannot be considered novel or involve an inventive step when	the document is taken alone								
citation	n or other special reason (as specified) ent referring to an oral disclosure, use, exhibition or	"Y" document of particular relevance cannot be considered to involve document is combined with on	e an inventive step when the								
other r	neans only published prior to the international filling date but	ments, such combination being	g obvious to a person skilled								
later th	an the priority date claimed	*&* document member of the same									
Date of the	actual completion of the international search	Date of mailing of the internation	onal search report								
19	9 January 2001	30.01.01									
Name and n	nailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2	Authorized officer									
	NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl.										
	Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016 Cupido, M										

## INTERNATIONAL SEARCH REPORT

in ational Application No

		PC1/EP 00/0/835
C.(Continu Category °	intion) DOCUMENTS CONSIDERED TO BE RELEVANT  Citation of document, with indication, where appropriate, of the relevant passages	Deliver to delegate
Category -	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LI X ET AL: "Mutation of lysine 405 to serine in the parvovirus H-1 NS1 abolishes its function for viral DNA replication, late promoter activation, and cytotoxocity"	1,3,7, 9-11
	JOURNAL OF VIRGLORY, vol. 64, no. 10, October 1990 (1990-10), pages 4654-4660, XP000867496 AMERICAN SOCIETY FOR MICROBIOLOGY US page 4656 -page 4657	
Α	J P F NÜESCH ET AL: "Sequence motifs in the replicator protein of parvovirus MVM essential for nicking and covalent attachement to the viral origin: identification of the linking tyrosine" VIROLOGY, US, ACADEMIC PRESS, ORLANDO, vol. 209, no. 1, 10 May 1995 (1995-05-10), pages 122-135, XP002088311 ISSN: 0042-6822 page 127 -page 131	1-13
A	S F COTTMORE ET AL: "The NS1 polypeptide of the murine parvovirus MVM binds to DNA sequences containing the motif (ACCA)2-3" JOURNAL OF VIROLOGY, US,THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 69, no. 3, pages 1652-1660, XF002088309 ISSN: 0022-538X page 1658, left-hand column, last paragraph -right-hand column	1-13



PCT/EP 00/07835

B x I Observati ns wh re c rtain claims wer f und unsearchable (Continuation of it m 1 f first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims is and 15 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the NS1 variant.
Claims Nos.:     because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically.
Claims Nos.:  because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.  .
As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
No required additional search fees were timely paid by the applicant. Consequently, this international Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.